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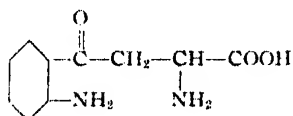
*HYDROXYANTHRANILIC ACID AS A PRECURSOR OF NICOTINIC ACID IN NEUROSPORA**

BY HERSCHEL K. MITCHELL AND JOSEPH F. NYC

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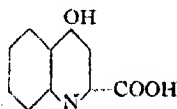
Communicated by G. W. Beadle, November 17, 1947

Recent investigations in this laboratory¹ have provided evidence that the biosynthesis of nicotinic acid in *Neurospora* proceeds from tryptophane through the intermediate kynurenine I.

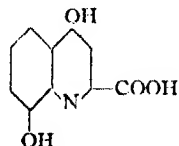


Kynurenine I

Further studies suggested that the pyridine ring of nicotinic acid might arise by ring closure of the α keto acid corresponding to kynurenine to give the naturally occurring compound kynurenic acid II² or, if preceded by oxidation, xanthurenic acid III.³



Kynurenic acid II

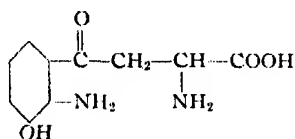


Xanthurenic acid III

In addition to these two compounds a series of nicotinic acid derivatives was synthesized and tested for growth promoting or growth inhibiting properties on *Neurospora* mutant 65001.¹ These compounds included: 3-carboxy-4-hydroxy pyridine, 3-carboxy-4-amino pyridine, 2-hydroxy-3-carboxy pyridine, 3-carboxy-6-hydroxy pyridine, 2,3-dicarboxy pyridine, 3,4-dicarboxy pyridine, 2,6-dimethyl-3,4-dicarboxy pyridine, 2,3,4-tricarboxy-6-methyl pyridine, 3-carboxy-4-chloro pyridine and 2,6-dimethyl-3-carboxy-4-chloro pyridine. In high concentrations, the compound 3-

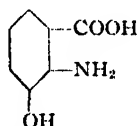
carboxy-4-amino pyridine promoted a small amount of growth, but the remaining compounds possessed no stimulatory or inhibitory action under the conditions utilized.

From the above facts it was concluded that the pyridine ring of nicotinic acid does not arise from kynurenine through kynurenic acid II or xanthurenic acid III followed by oxidation of the benzene ring. It also appeared evident that the oxidation in position 8 of xanthurenic acid III precedes formation of the pyridine ring, a possible intermediate being 3-hydroxy-kynurenine IV.



Hydroxykynurenine IV

A consideration of this hypothetical compound suggested the possibility of biological oxidation to give 3-hydroxyanthranilic acid (2-amino-3-hydroxybenzoic acid) V instead of xanthurenic acid.



Hydroxyanthranilic acid V

A trimethyl derivative of this compound V is indeed found in nature as the alkaloid damascanine (2-methyl-amino-3-methoxy-methyl benzoate).^{4,5} The alkaloid has been isolated from the seeds of two species of *Nigella* (common name of flowers, Love in a Mist).

It is the purpose of the experimental part of the present paper to present evidence that hydroxyanthranilic acid is an intermediate in the biological synthesis of nicotinic acid from tryptophane in *Neurospora*.

Hydroxyanthranilic acid has been synthesized in this laboratory by two independent methods. These methods and the proof of structure of the active compound will be presented elsewhere.

Experimental.— Media and conditions for growth of mutant 65001 have been previously described.^{1,6} Growth curves for this mutant in the presence of nicotinamide and hydroxyanthranilic acid (filter sterilized) are presented in figure 1. For these experiments the pH of the medium was adjusted to 4.1 since hydroxyanthranilic acid, like nicotinic acid, is less active at a higher pH where dissociation is greater. In four days at a pH of 5.6 the compound is 50 to 70% as effective as nicotinamide in promoting growth. It is thus more effective than nicotinic acid at pH 5.6.⁷

The growth-promoting activity of hydroxyanthranilic acid on several

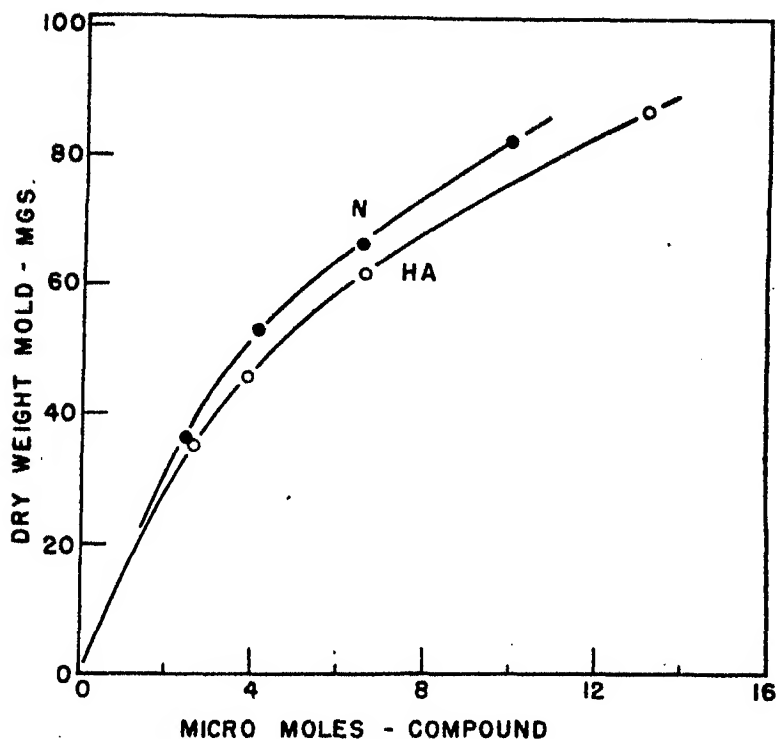


FIGURE 1

Growth curves of mutant 65001 (8½ days) in the presence of nicotinamide (curve N) and hydroxyanthranilic acid (curve HA).

genetically different mutants of *Neurospora* was compared to that of anthranilic acid, indole, tryptophane, kynurenine and nicotinamide, and qualitative data is presented in table 1.

TABLE 1

ACTIVITY OF HYDROXYANTHRANILIC ACID COMPARED TO ANTHRANILIC ACID, INDOLE, TRYPTOPHANE, KYNURININE AND NICOTINAMIDE ON *Neurospora* MUTANTS

MUTANT STRAIN	ANTHRANILIC ACID	INDOLE	TRYPTOPHANE	KYNURININE	HYDROXY-ANTHRANILIC ACID	NICOTINAMIDE
44008	-	+	+	+	+	+
65001	-	+	+	+	+	+
39401	-	+	+	+	+	+
4540	-	-	-	-	-	+
3416	-	-	-	-	-	+

It was previously shown¹ that an excess of a compound with nicotinamide activity is produced by mutant 65001 when it is grown in the presence of an

excess of kynurenine. Similar experiments with hydroxyanthranilic acid are summarized in table 2. Nicotinic amide activity was determined by use of strain 3416 which does not utilize hydroxyanthranilic acid. Determinations were made on culture fluids of six-day-old cultures of 65001 and 44008 grown in the presence of various quantities of hydroxyanthranilic acid.

TABLE 2
PRODUCTION OF NICOTINAMIDE ACTIVITY FROM HYDROXYANTHRANILIC ACID BY MUTANTS 65001 AND 44008

HYDROXYANTHRANILIC ACID, μ G. PER 20 ML.	DRY WEIGHT		NICOTINAMIDE ACTIVITY, μ G. PER 20 ML. OF CULTURE FLUID	
	65001	44008	65001	44008
0	2	0	0	0
20	84	97	0	0
50	97	95	0	0
100	108	94	10	12
200	103	85	12	12

Discussion.—It is evident from the experimental data presented, that for certain *Neurospora* mutants, hydroxyanthranilic acid possesses growth-promoting activity that is quite comparable to the activity of nicotinamide. In addition it has been demonstrated that in the presence of an excess of hydroxyanthranilic acid two of the *Neurospora* mutants produce an excess of a substance with the biological activity of nicotinic acid or nicotinamide. This was determined by use of a mutant that utilizes either of the latter two compounds but does not utilize hydroxyanthranilic acid.

Thus it appears probable that this substance is a natural intermediate in the biological synthesis of nicotinic acid by the mold *Neurospora*. It is of interest to note the complex series of reactions that are required by the mold to convert anthranilic acid to hydroxyanthranilic acid. These reactions are illustrated schematically in figure 2.

No comparison has been made, in this laboratory, between the properties of hydroxyanthranilic acid and those of the nicotinic acid precursor from *Neurospora* described by Bonner and Beadle.⁷ From the published data it can be seen that the molecular formula is similar. The isolated precursor, however, is reported to be a pyridine derivative. As such it would be expected to be further along in the series of reactions leading to nicotinic acid synthesis. In this connection it may be suggested that hydroxyanthranilic acid can be converted to nicotinic acid by oxidation and loss of carbon three of the compound, followed by ring closure or rearrangement in the six carbon amino acid residue. If this occurs in animals and in *Neurospora* the 3-carboxy-6-pyridone isolated by Knox and Grossman⁸ may well be a by-product of the reaction. Similarly, the occurrence of damascanine in *Nigella* may be accounted for as resulting from a side reaction in the biosynthesis of nicotinic acid in the organism.

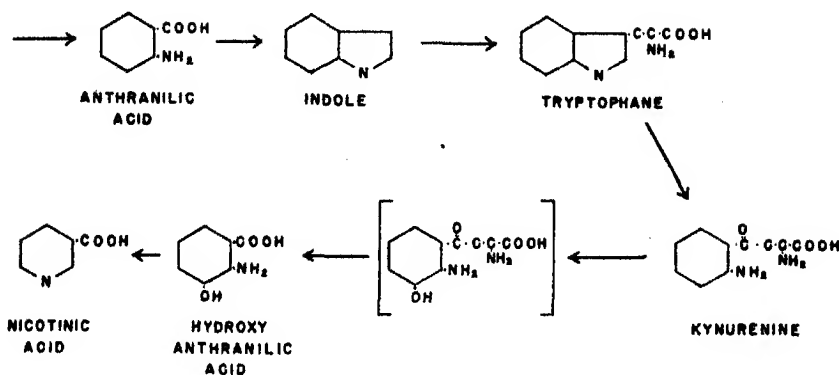


FIGURE 2

A schematic representation of a series of reactions leading to the biosynthesis of nicotinic acid in *Neurospora*.

Summary.—1. Evidence is presented to show that hydroxyanthranilic acid (2-amino-3-hydroxy benzoic acid) is an intermediate in the biosynthesis of nicotinic acid in *Neurospora*.

2. Several nicotinic acid derivatives and other related compounds are shown to lack significant biological activity.

* These investigations were supported by funds from the Rockefeller Foundation and the Williams-Waterman Fund for the Combat of Dietary Diseases.

¹ Beadle, G. W., Mitchell, H. K., and Nye, J. F., *Proc. Nat. Acad. Sci.*, **33**, 155 (1947).

² Ellinger, A., *Ber.*, **37**, 1801 (1904).

⁸ Lepkovsky, S., Roboz, E., and Haagen-Smit, A. J., *J. Biol. Chem.*, **149**, 195 (1943).

⁴ Pommerehne, F., *Archiv. Pharm.*, **238**, 531 (1900).

⁵ Keller, O., *Ibid.*, 246, 1 (1908).

⁶ Beadle, G. W., and Tatum, E. L., *Am. J. Bot.*, **32**, 678 (1945).

⁷ Bonner, D. M., and Beadle, G. W., *Archiv. Biochem.*, **11**, 319 (1946).

^a Knox, W. E., and Grossman, W. I., *J. Biol. Chem.*, **166**, 391 (1946).

THE IDENTIFICATION OF A NATURAL PRECURSOR OF NICOTINIC ACID*

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Communicated by E. W. Sinnott, December 11, 1947

In a previous paper¹ the production and isolation of a natural precursor of nicotinic acid was described. The present paper deals with the identification of this precursor.

Several mutant strains of *Neurospora crassa* have been characterized as

requiring nicotinic acid, nicotinamide, or related compounds for growth.^{1, 2} Genetic investigation of these strains indicates at least three genetic types¹ which in accord with the usual interpretation³ suggests at least three separate steps in the biosynthesis of nicotinic acid. Since in theory different biosynthetic steps are blocked in the various mutant strains requiring nicotinic acid for growth, culture filtrates were tested for the accumulation of intermediates. Strain #4540 when grown in limiting amounts of nicotinamide was found to accumulate a substance possessing nicotinic acid activity for a strain of a second genetic type (#39401).¹ Fractionation of culture filtrates yielded a small amount of a crystalline compound as active as nicotinic acid for growth of strain #39401.¹

Elementary analysis of the isolated material establishes the probable empirical formula $C_7H_7O_2N$. Due to the difficulty encountered in preparing sufficient amounts of pure substance, however, analysis of material of unquestionable purity has not been carried out.

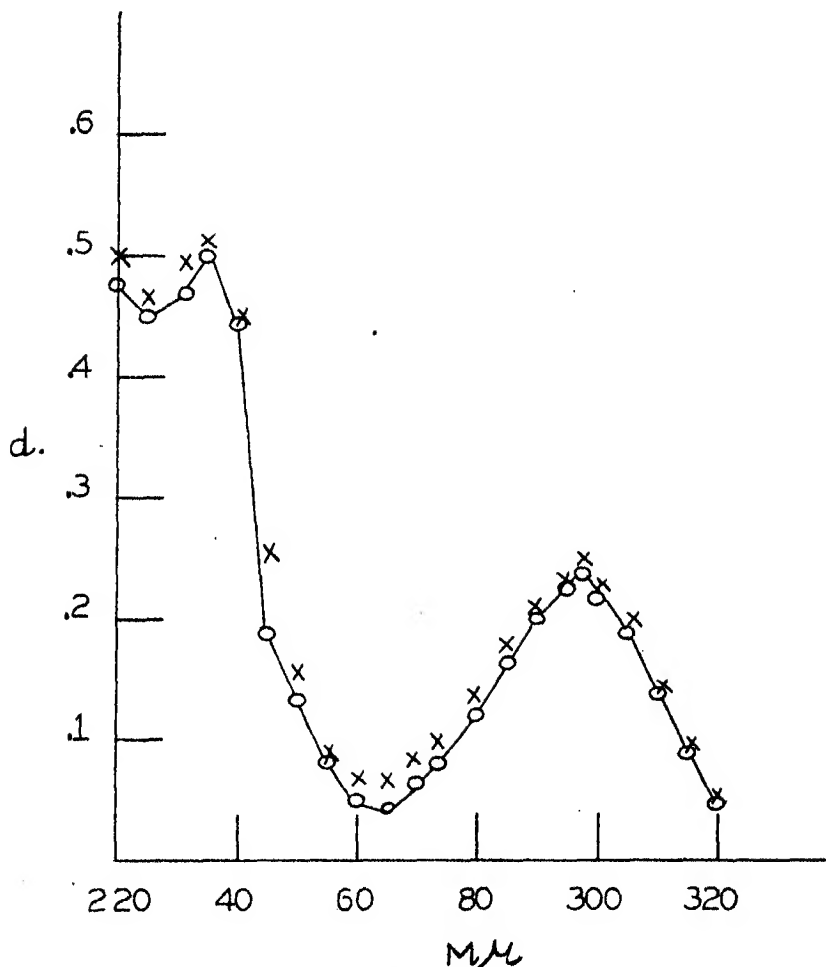
	% C	% H	% N
Calculated for $C_7H_7O_2N$	54.8	4.6	9.2
Found	54.6	4.8	10.1

Determination of the molecular weight suggests either a C-6 or C-7 structure. Since the physical properties of the isolated material resembled those of the pyridone carboxylic acids, several pyridones were prepared. The 6-oxy-nicotinic acid, 4-oxy-nicotinic acid, 2-oxy-nicotinic acid, 4 amino-nicotinic acid, and 2,3-dicarboxy pyridine were prepared, tested and found inactive. In addition samples of N-methyl-6-oxy-nicotinic acid, and N-methyl-6-oxy-nicotinamide¹ were also tested and found inactive. Following a different approach in their investigation of the biosynthesis of nicotinic acid Mitchell and Nyc⁴ prepared 3-hydroxy-anthranilic acid (2-amino, 3-hydroxy benzoic acid) and found it active as a precursor of nicotinic acid for strain #39401. Comparison of the physical and biological properties of the precursor isolated from *Neurospora* culture filtrates with a sample of 3-hydroxy-anthranilic acid, generously supplied by Drs. H. K. Mitchell and J. F. Nyc, California Institute of Technology, indicates identity of these two compounds. Table 1 lists the melting point and sublimation behavior of the two compounds, and figure 1 shows a comparison of the absorption spectra of the two compounds at a concentration of 10 γ /cc. 1M HCl from 320 $m\mu$ to 230 $m\mu$.

TABLE 1

A COMPARISON OF THE PHYSICAL PROPERTIES OF THE PRECURSOR ISOLATED FROM *NEUROSPORA* FILTRATES, AND OF 3-HYDROXY-ANTHRANILIC ACID

	ISOLATED	SYNTHETIC
Melting point	255°C.-d.vig. gas evolution	255°C.-d.vig. gas evolution
Mixed melting point	255°C.-d.vig. gas evolution	255°C.-d.vig. gas evolution
Sublimation <i>in vacuo</i>	170-180°C.	170-180°C.
Absorption maxima	297 and 235 $m\mu$	297 and 235 $m\mu$



activity of 3-hydroxy-anthranilic acid is the same as that of the isolated precursor for the growth of this mutant strain.

TABLE 2
GROWTH OF VARIOUS MUTANT STRAINS OF *NEUROSPORA* ON NICOTINIC ACID AND RELATED COMPOUNDS

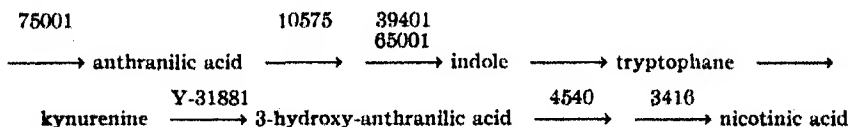
STRAIN NO.	ANTHRANILIC ACID	TRYPTOPHANE	KYNURENINE	ISOLATED PRECURSOR	3-HYDROXY-ANTHRANILIC ACID	NICOTINIC ACID
39401	—	+	+	+	+	+
65001	—	+	+	+	+	+
Y31881	—	—	—	+	+	+
4540	—	—	—	—	—	+
3416	—	—	—	—	—	+
75001	+	+	+	—	—	—
10575	—	+	—	—	—	—

The physical properties and biological activity of the precursor prepared from *Neurospora* filtrates appear, therefore, to be identical with those of 3-hydroxy-anthranilic acid. On the basis of these comparisons, the isolated *Neurospora* precursor is assigned the structure, 3-hydroxy-anthranilic acid (2-amino 3-hydroxy-benzoic acid).

Discussion.—With the identification of the *Neurospora* precursor as 3-hydroxy-anthranilic acid, the scheme of biosynthesis of nicotinic acid previously proposed would appear as:



From work of Beadle, *et al.*,² kynurenine, tryptophane and the tryptophane precursor indole are known to replace nicotinic acid for strains 39401 and 65001. No strain has been found to date which can utilize anthranilic acid in place of nicotinic acid. This might suggest the sequence:



Such a scheme has been suggested by Beadle, *et al.*,² and by Mitchell and Nyc.⁴ There are, however, certain discrepancies which are difficult to reconcile with such an interpretation. Mutant strains are known which accumulate anthranilic acid⁵ yet which give no growth response to nicotinic acid, and there are also mutant strains known which can use tryptophane, indole, anthranilic acid or kynurenine which cannot utilize hydroxy-anthranilic acid or nicotinic acid for growth⁶ (see table 2). Using appropriate genetic stocks it has also been impossible to detect the conversion of

tryptophane or kynurenine to 3-hydroxy-anthranilic acid. The inactivity of kynurenine in replacing tryptophane or nicotinic acid as a precursor of *N*'-methyl nicotinamide in the rat has been reported by Rosen, *et al.*⁶ Both kynurenine and 3-hydroxy-anthranilic acid have been found inactive in replacing tryptophane and nicotinic acid in preliminary growth experiments with rats.⁷ It should be pointed out, therefore, that while these compounds are related to nicotinic acid synthesis, the specific rôle of each compound cannot as yet be definitely assigned.

Summary.—On the basis of the similarity in the physical and biological properties of a natural precursor of nicotinic acid isolated from *Neurospora* filtrates, and of 3-hydroxy-anthranilic acid, it is concluded that these two compounds are identical.

¹ Bonner, D., and Beadle, G. W., *Archiv. Biochem.*, **11**, 319 (1946).

² Beadle, G. W., Mitchell, H. K., and Nyc, J. F., *Proc. Nat. Acad. Sci.*, **33**, 155 (1947).

³ Bonner, D., *Cold Spring Harbor Symp. Quant. Biol.*, **11**, 14 (1946).

⁴ Mitchell, H. K., and Nyc, J. F., these PROCEEDINGS, **34**, 1-5 (1948).

⁵ Tatum, E. L., Bonner, D., and Beadle, G. W., *Archiv. Biochem.*, **3**, 477 (1944).

⁶ Rosen, F., Huff, J. W., and Perlzweig, W. A., *J. Nutrition*, **33**, 561 (1947).

⁷ Krehl, W. A., and Bonner, D., unpublished.

* These investigations were supported in part by a grant from the Williams-Waterman Fund for the Combat of Dietary Diseases.

† Generously supplied by Dr. W. Eugene Knox, College of Physicians and Surgeons, Columbia University.

ON LINE CONGRUENCES

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1. This paper summarizes without proofs some results on the following problem in projective three-dimensional space.

Let a two-parameter family of curves γ be given such that through every point of some region R of three-space there passes one and only one curve γ . Moreover consider a p -parameter family of surfaces Σ , such that in R every curve γ and every surface Σ have precisely one point in common. Then the family of curves γ determines in R a one-to-one correspondence between any two of the surfaces Σ . Now, assuming that for each pair of surfaces Σ this correspondence is *asymptotic*, i.e., maps the asymptotic lines of the one surface onto those of the other, we ask for the maximal value of p and furthermore we want to determine those congruences of

curves γ which admit p -parameter families of surfaces Σ with maximal value of p . We suppose that none of the surfaces Σ contains only one one-parameter family of asymptotic lines, i.e., is developable without being a plane (planes will be looked upon as developable surfaces).

2. Evidently the three-parameter family of planes in three-space is a p -parameter family of surfaces Σ for any congruence of curves γ . Consequently the maximal value of p is at least three. However, it is easy to see that p can have at least the value four. For that purpose let us consider the congruence of straight lines γ through a fixed point O . There exists a four-parameter family G of projectivities (containing a three-parameter subfamily of *singular* projectivities), each of whose members maps any of the lines γ into itself (provided for the singular members of G we disregard the point O of γ). Hence, if Σ_0 is an arbitrary non-developable surface not containing O , then the four-parameter family of surfaces Σ , obtained by subjecting Σ_0 to all projectivities of G , possesses the desired property with respect to the congruence of lines γ through O . Apparently the four-parameter family of surfaces Σ is not uniquely determined by the congruence of lines γ but depends on one arbitrary function of two variables. The congruence of lines γ depends on three arbitrary constants. Apparently all nonplanar surfaces Σ of the same four-parameter family are projectively equivalent.

3. As a first step in the solution of the problem it can be proved that the maximal value of p cannot exceed four. Moreover, it turns out that, if a congruence of curves γ admits a four-parameter family of surfaces Σ , the curves γ have to be straight lines. In this case the developable surfaces of the congruence intersect every surface Σ in a conjugate net.

In view of this result it is convenient to consider the following types of congruences of straight lines γ :

I. The congruence is not parabolic and at least one of its focal surfaces is not a curve.

II. The congruence is parabolic and its focal surface is not a curve.

III. The congruence is not parabolic and both focal surfaces are curves.

IV. The congruence is parabolic and its focal surface is a curve.

V. The congruence consists of all straight lines through a fixed point.

Case V was already taken care of in §2.

4. Before stating the results for a congruence of type I we recall that a conjugate net on a surface is said to be an R -net, if and only if the two congruences constituted by the tangents of the curves of each of both one-parameter families of this net are W -congruences, i.e., congruences of which the asymptotic lines of the one focal surface correspond to those of the other. It is well known that an R -net is isothermally conjugate.

It can be proved that a congruence of type I admits a four-parameter family of surfaces Σ if and only if its developable surfaces intersect one of

the focal surfaces (and therefore also the other) in an R -net. Such a congruence depends on six arbitrary functions of one variable.

In this case the family of surfaces Σ is uniquely determined by the congruence. Their asymptotic lines not only correspond to each other but also to those of the focal surfaces. The developable surfaces of the congruence not only intersect each of the focal surfaces but also each of the surfaces Σ in an R -net.

5. A congruence of type II admits a four-parameter family of surfaces Σ if and only if its focal surface has a projective line-element which can be written in the form

$$\frac{du^2 + \gamma dv^2}{2 dudv},$$

such that the congruence consists of the tangents to the asymptotic lines of the focal surface represented by the equation $v = \text{constant}$, γ being some function of u and v . Such a congruence depends on five arbitrary functions of one variable.

The family of surfaces Σ is uniquely determined by the congruence. The asymptotic lines of the surfaces not only correspond to each other but also to those of the focal surface. Each surface Σ has a projective line-element of the form

$$\frac{du^2 + \gamma_2 dv^2}{2 dudv},$$

where u and v are the same coordinates as those mentioned before. The function γ_2 depends on the choice of Σ .

6. If the congruence is of type III, then it admits a four-parameter family of surfaces Σ if and only if its focal curves are two skew straight lines. Apparently this congruence depends on eight arbitrary constants.

The family of surfaces Σ is not uniquely determined by the congruence but depends on two arbitrary functions of one variable, or, more precisely, every non-developable surface, on which the two pencils of planes through the two focal lines intersect a conjugate net, belongs to one and only one four-parameter family of surfaces Σ .

The conjugate net which is the intersection of the two above-mentioned pencils of planes with any surface Σ is an R -net. Each of the two families which together constitute the net consists of projectively equivalent plane curves.

7. A congruence of type IV admits a four-parameter family of surfaces Σ if and only if it consists of a one-parameter family of pencils of straight lines γ such that the vertices of these pencils do not coincide and such that the plane of every pencil osculates the locus of vertices at the vertex

of this pencil. Apparently this congruence depends on two arbitrary functions of one variable.

The four-parameter family of surfaces Σ is not uniquely determined by the congruence but depends on two arbitrary functions of one variable, or more precisely, every non-developable ruled surface, whose generators are contained in the planes of the above-mentioned pencils but do not belong to these pencils, belongs to one and only one four-parameter family of surfaces Σ .

Every surface Σ is a ruled surface whose generators are contained in the planes of the pencils but do not belong to these pencils.

If we compare this case with the others (particularly with types III and V), then it shows a remarkable irregularity with respect to the data required for the determination of the congruence: whereas congruences of types III and V depend on constants only, a congruence of type IV depends on two arbitrary functions of one variable.

8. The results of §4 can be completed in the following way. If, for some non-parabolic W -congruence of which at least one focal surface is not a curve, there exists a non-developable surface S , which does not coincide with either focal surface and whose asymptotic lines correspond to those of the focal surfaces, then the developable surfaces of that congruence intersect each of the focal surfaces in an R -net. Hence, according to §4, the existence of one surface S implies the existence of a four-parameter family of surfaces S . These surfaces S , together with the three-parameter family of planes, constitute the four-parameter family of surfaces Σ mentioned in §4.

A similar result can be added to §5. If, for some parabolic W -congruence whose focal surface is not a curve, there exists a non-developable surface S which does not coincide with the focal surface and whose asymptotic lines correspond to those of the focal surface, then the line-element of the focal surface of that congruence has the form mentioned in §5. Consequently, the existence of one surface S implies the existence of a four-parameter family of surfaces S . Together with the three-parameter family of planes these surfaces S constitute the four-parameter family of surfaces Σ mentioned in §5.

A SIMPLE SECOND ORDER DIFFERENTIAL EQUATION WITH SINGULAR MOTIONS

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It has been announced by Cartwright and Littlewood¹ that the equation

$$\frac{d^2x}{dt^2} + k(x^2 - 1) \frac{dx}{dt} + x = bk\lambda \cos(\lambda t + \alpha)$$

for large k and certain values of b has singular motions. (These are discontinuous recurrent in the sense of Birkhoff.) A sketch of their method is given but the detailed account has not yet appeared.

Here we shall give an example of an equation with singular motions for which the proof is decidedly simpler. The equation is

$$\frac{d^2y}{dt^2} + P(y) \frac{dy}{dt} + y = c \sin t,$$

where $P(y)$ is a polynomial and c is a constant.

The device we use is to consider first the equation

$$\epsilon \frac{d^2x}{dt^2} + \phi(x) \frac{dx}{dt} + \epsilon x = b \sin t, \quad (1)$$

where $\epsilon > 0$ is a small constant and b , $0 < b < 1$, is a constant, and where $\phi(x) = 1$, $|x| > 1$, and $\phi(x) = -1$, $|x| < 1$. We introduce

$$\rho = [1 - (1 - 4\epsilon^2)^{1/2}]/(2\epsilon) = \epsilon + \epsilon^3 + \dots$$

For $|x| > 1$ and for $|x| < 1$ the equation (1) is linear with constant coefficients and can of course be solved explicitly. We consider any continuous family of solutions starting with $t = \pi + \tau$ and satisfying

$$x = -1, \quad \frac{dx}{dt} = \rho(1 + b \cos \tau) - b \sin \tau + E, \quad \left| \tau - \frac{\rho}{b} \right| \leq k\rho,$$

where k is a small constant and E is any constant satisfying $|E| \leq e^{-1/\epsilon}$. As t increases these solutions all rise above $x = 2.9$ and then gradually decrease. They pass downward across $x = 1$ over a range in t which we can regard as a map of the original interval. This map includes the two intervals $t = 2n\pi + \tau$ and $t = 2(n+1)\pi + \tau$ where $|\tau - \rho/b| \leq 2k\rho$ and n is a large integer. Moreover x satisfies a relationship similar to the original with E now satisfying $|E| \leq 1/2e^{-1/\epsilon}$. Each interval $|\tau - \rho/b| \leq k\rho$ contained in each of the two intervals on $x = 1$ then maps out two others on $x = -1$, etc.

That is, if we denote the distance between the original τ interval and its two images on $x = 1$, which are close to $(2n - 1)\pi$ and $(2n + 1)\pi$, by the erroneous designation "semi-periods" choosing at each step either $(2n - 1)\pi$ or $(2n + 1)\pi$, then there is a motion starting in the original τ interval that has these prescribed "semi-periods." This is the device used by Cartwright and Littlewood but because (1) can be solved explicitly the result here is easy to demonstrate.

The objection to our procedure so far is that ϕ is discontinuous rather than analytic. Consider the system

$$\epsilon \frac{dx_1}{dt} = x_2 - \Phi(x_1), \quad \frac{dx_2}{dt} = -\epsilon x_1 + b \sin t \quad (2)$$

where $\Phi(x) = \int_0^x \phi(x) dx$. Then eliminating x_2 we see that x_1 is x of (1).

It is easy to show that for the family of solutions of (1) under consideration, $|x| < 5$. Let $P(x)$ be a polynomial such that for $|x| \leq 6$, $|P(x) - \Phi(x)| \leq \eta$ where η is small. This is possible since $\Phi(x)$ is continuous. Consider the system

$$\epsilon \frac{dy_1}{dt} = y_2 - P(y_1), \quad \frac{dy_2}{dt} = -\epsilon y_1 + b \sin t. \quad (3)$$

If $y = y_1$ then

$$\epsilon \frac{d^2 y}{dt^2} + P'(y) \frac{dy}{dt} + \epsilon y = b \sin t. \quad (4)$$

The first equation of (3) can be written as

$$\epsilon \frac{dy_1}{dt} = y_2 - \Phi(y_1) + s$$

where $|s| \leq \eta$. Since Φ satisfies a Lipschitz condition with constant 1 we have if $y_i(\pi + \tau) = x_i(\pi + \tau)$, $i = 1, 2$, that

$$|y_1(t) - x_1(t)| + |y_2(t) - x_2(t)| \leq \frac{\eta}{1 + \epsilon^2} \epsilon^{[(\epsilon+1/\epsilon)|t-\pi-\tau| - 1]}.$$

By taking η small enough we can make $|y_1 - x_1|$ and $|y_2 - x_2|$ as small as we want. Thus $|y - x|$ and $|(dy/dt) - (dx/dt)|$ can be made arbitrarily small for $\pi + \tau \leq t \leq 2(n + 2)\pi$. This implies that the original τ interval undergoes the same kind of mapping for (4) as for (1). Thus the τ interval on $y = -1$ has an image on $y = 1$ which includes two τ intervals. The result just demonstrated can then be applied to each of these two, etc.

Thus we have motions for y with a preassigned infinite sequence of "semi-periods" $(2n - 1)\pi$ or $(2n + 1)\pi$. There are in fact motions originating in the τ interval with preassigned past and future "semi-periods."

The existence of motions determined by arbitrary sequences of numbers

has been demonstrated in classical mechanics for systems with two degrees of freedom by G. D. Birkhoff.²

If we consider the transformation T of the (y, \dot{y}) plane into itself effected by the motions of (4) after an elapse of t equal to 2π , then there is a fixed point, P_0 , under T representing an unstable periodic motion of period 2π (corresponding to $x = b \cos t$ in the x case). There is an invariant "curve" C which is really a closed connected set of zero area separating P_0 from the remote part of the (y, \dot{y}) plane. All points, except P_0 , tend to C under T . (The existence of C follows easily from the fact that area away from P_0 in the (y, \dot{y}) plane eventually decreases as t increases.) C must be complicated because the rotation numbers of its accessible exterior and interior points clearly cannot determine the multiplicity of rotation numbers of those singular motions which have rotation numbers.

¹ Cartwright, M. L., and Littlewood, J. E., On Non-Linear Differential Equations of the Second Order: 1. The Equation $\ddot{y} - k(1 - y_1)\dot{y} + y = b\lambda k \cos(\lambda t + \alpha)$, k Large. *J. London Math. Soc.*, 20, 180-189 (1945).

² Birkhoff, G. D., Sur Le Probleme Restreint Des Trois Corps, 11^e Memoire, *Ann. R. Scuola Normale Superiore di Pisa*, Ser II, 5, 9-50 (1936).

THE NUMBER OF ZEROS OF A POLYNOMIAL IN A CIRCLE

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Communicated by J. L. Walsh, November 26, 1947

In this note two new theorems will be stated concerning the number p of zeros of a real or complex polynomial in a circle C , which for convenience' sake will be assumed to be the circle $|z| = 1$. The first theorem, as will be indicated below, leads to a proof of the well-known Schur-Cohn criterion for p , a proof without the customary use of Hermitian forms.

THEOREM I. Let $f(z) = \sum_{k=0}^n a_k z^k$ and $f^*(z) = \sum_{k=0}^n \bar{a}_k z^{n-k}$, where \bar{a}_k denotes the conjugate imaginary of a_k . Let the sequence of polynomials $f_j(z) = \sum_{k=0}^{n-j} a_k^{(n-j)} z^k$ be defined by recursion formula

$$f_{j+1}(z) = \bar{a}_0^{(j)} f_j(z) - a_{n-j}^{(j)} f_j^*(z), \quad j = 0, 1, \dots, n-1$$

with $f_0(z) = f(z)$. If $\delta_j = f_j(0) \neq 0$ for $j = 1, 2, \dots, n$, then $f(z)$ has no zeros on C and p zeros in C , p being the number of negative products $P_j = \delta_1 \delta_2 \dots \delta_j$, $j = 1, 2, \dots, n$.

The proof of Theorem I is based upon the

LEMMA. If $f_j(z)$ has p_j zeros in C and none on C , the $f_{j+1}(z)$ has

$$p_{j+1} = (1/2)\{n - j - [(n - j) - 2p_j]sg \delta_{j+1}\} \quad (1)$$

zeros in C and none on C .

In this lemma, $sg x$ denotes the sign of the real number x .

The lemma may be proved by using Rouché's Theorem or by combining two lemmas due to Cohn.¹

Iteration of formula (1) produces the equation

$$p_j = (1/2)[(n - j + 1) - (n - p)sg P_j + \sum_{i=1}^{j-1} sg(P_j/P_i)]. \quad (2)$$

From this it follows that, since the degree of $f_n(z)$ is zero,

$$0 = p_n = (1/2)[1 - (n - 2p)sg P_n + \sum_{i=1}^{n-1} sg(P_n/P_i)]. \quad (3)$$

The proof of Theorem I is completed by solving equation (3) for p .

Under the hypotheses of Theorem I, no $f_j(z)$, $j \leq n$, may be identically zero. An extension to cover the contrary case is expressed in

THEOREM II. *If for some $k < n$ $f_{k+1}(z) \equiv 0$ but $P_k \neq 0$, then $f(z)$ has $n - k$ zeros on C and p zeros inside C , p being the number of negative P_j , $j \leq k$.*

The hypothesis $f_{k+1}(z) \equiv 0$ implies that $f(z)$ and $f^*(z)$ have an $(n - k)$ -degree common factor $h(z)$ which has all its zeros on C . Theorem II is proved essentially by applying Theorem I to the polynomial $g(z) = f(z)/h(z)$.

Theorem I will now be applied to proving the Schur-Cohn criterion¹ which may be stated as follows.

THEOREM III. *Let Δ_k denote the determinant of order $2k$*

$$\begin{vmatrix} a_0 & 0 & 0 & \dots & 0 & a_n & a_{n-1} & \dots & a_{n-k+1} \\ a_1 & a_0 & 0 & \dots & 0 & 0 & a_n & \dots & a_{n-k+2} \\ \vdots & \vdots & \vdots & \dots & \vdots & \vdots & \vdots & \dots & \vdots \\ a_{k-1} & a_{k-2} & a_{k-3} & \dots & a_0 & 0 & 0 & \dots & a_n \\ \bar{a}_n & 0 & 0 & \dots & 0 & \bar{a}_0 & \bar{a}_1 & \dots & \bar{a}_{k-1} \\ \bar{a}_{n-1} & \bar{a}_n & 0 & \dots & 0 & 0 & \bar{a}_0 & \dots & \bar{a}_{k-2} \\ \vdots & \vdots & \vdots & \dots & \vdots & \vdots & \vdots & \dots & \vdots \\ \bar{a}_{n-k+1} & \bar{a}_{n-k+2} & \bar{a}_{n-k+3} & \dots & \bar{a}_n & 0 & 0 & \dots & \bar{a}_0 \end{vmatrix}.$$

If $\Delta_k \neq 0$ for $k = 1, 2, \dots, n$, then $f(z)$ has no zeros on C and p zeros inside C , p being the number of variations of sign in the sequence $1, \Delta_1, \Delta_2, \dots, \Delta_n$.

If $\Delta_k^{(j)}$ denotes the corresponding determinant for the coefficients $a_k^{(j)}$, the reduction formula

$$\Delta_k^{(j)} = \Delta_{k-1}^{(j+1)} / (\delta_{j+1})^2$$

may be established and, when applied to Δ_k , leads to the equation

$$\Delta_k = \delta_k / (\delta_1^{k-2} \delta_2^{k-3} \dots \delta_{k-2}).$$

This means that $sg(\Delta_k \Delta_{k+1}) = sg P_{k+1}$ and thus completes the proof of Theorem III.

Further details concerning the proofs and implications of the theorems will be published elsewhere.

¹ Cohn, A., *Math. Zeitschr.*, **14**, 110-148 (1922).

INFINITE DIMENSIONAL DIFFERENTIAL METRICS WITH CONSTANT CURVATURE

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Communicated by T. Y. Thomas, September 29, 1947

1. *Introduction.*—Some general theorems on infinite dimensional differential metrics with constant curvature were given by the author¹ some time ago. In this paper we shall give some more recent special results including a few on infinite dimensional and/or dimensionless Hermitian differential metrics.² The author's¹⁴ more general results for a theory of infinite dimensional hypersurfaces of revolution will not be given here. These studies include a theory of the generalized second fundamental form of hypersurfaces, and the abstract Gauss and Mainardi-Codazzi equations. These are the equations that relate a hypersurface to its enveloping infinitely dimensional $(\infty + 1)$ space.

2. *Hyperspheres of Composite Abstract Euclidean Spaces.*—Let E be an abstract (real) Euclidean space³ with Banach norm $\|y\|$ and independently postulated positive definite real inner product $[y, z]$. The "Hilbert space relation" $\|y\| = [y, y]^{1/2}$ does *not necessarily* hold. By a composite abstract Euclidean space E_c we shall mean the abstract Euclidean space of all points (y_0, y) with y_0 real and y in E such that the norm is defined by

$$\|(y_0, y)\| = (y_0^2 + \|y\|^2)^{1/2} \quad (1)$$

(or by any other equivalent Banach norm) while the inner product is taken as

$$y_0 z_0 + [y, z]. \quad (2)$$

The differential metric of E_c will be

$$ds^2 = (dy_0)^2 + [dy, dy] \quad (3)$$

while the equation of the *hypersphere* in E_c with radius R and center at $(0, 0)$ will be taken as

$$y_0^2 + [y, y] = R^2. \quad (4)$$

It can be shown that as the parameter x ranges over E the equations

$$y_0 = \frac{1}{\sqrt{K}} \left(\frac{2}{u} - 1 \right), \quad y = \frac{x}{u} \quad (5)$$

define a parametric representation of the hypersphere (4) in E_C if $K = 1/R^2$, $u = 1 + (K/4)\rho^2$ and $\rho^2 = [x, x]$. In fact (5) is a *generalization of stereographic projection* of classical geometry.

The differential metric of the hypersphere (4) of E_C can with the aid of (5) be shown to be

$$ds^2 = \frac{[dx, dx]}{\{1 + (K/4)[x, x]\}^2}. \quad (6)$$

Hence from the author's general theory, we see that the hypersphere (4) in E_C is a Michal-Riemann space⁴ of constant curvature $K = 1/R^2$.

Another differential metric of the hypersphere (4) of E_C can be obtained by a *generalized orthogonal projection of the hypersphere (4) of E_C on the "equatorial plane" $y_0 = 0$* . For points not on the "equatorial plane," i.e., for points y in E such that $1 - K[y, y] \neq 0$, the result is

$$ds^2 = [dy, g(y, dy)], \quad (7)$$

where

$$g(y, \xi) = \xi + \frac{K[y, \xi]y}{1 - K[y, y]}. \quad (8)$$

Both (6) and (7) are positive definite differential forms.¹⁵

3. *Spaces of Positive, Negative and Zero Curvature.*¹⁶—Now let us start *ab initio* with the positive definite differential metric

$$ds^2 = [dy, g(y, dy)], \quad (9)$$

where

$$g(y, \xi) = \xi + \frac{k[y, \xi]y}{1 - k[y, y]} \quad (10)$$

and k can be positive, negative or zero. If $k > 0$, it is understood in (10) that y of E does not satisfy $1 - k[y, y] = 0$. It can be shown by a direct calculation and some well-known or easily proved theorems on Fréchet differentials that the *abstract⁵ Christoffel symbol of the second kind exists and is given by*

$$\Gamma(y, \xi_1, \xi_2) = k[\xi_1, g(y, \xi_2)]y. \quad (11)$$

From this it can be shown by a long but straightforward calculation that

the metric function $g(y, \xi)$ of the differential metric (9) satisfies the following abstract differential equation of the second order in the Fréchet differentials of $g(y, dy)$

$$R(y, d_1y, d_2y, d_3y) = k([d_1y, g(y, d_2y)]g(y, d_3y) - [d_1y, g(y, d_3y)]g(y, d_2y)) \quad (12)$$

for all y in E such that $1 - k[y, y] \neq 0$ and for all d_iy in E . In (12) the function $R(y, d_1y, d_2y, d_3y)$ is the abstract⁵ "Riemann-Christoffel" form based on the $g(y, dy)$ of the differential metric (9). It follows from (12) that the differential metric (9) is of constant curvature k irrespective of whether k is positive, negative or zero.

4. *Geodesics⁵ in Abstract Spaces of Constant Curvature $k \neq 0$.*—The second order abstract "ordinary" differential equation,

$$\frac{d^2y(s)}{ds^2} + ky(s) = 0 \quad (s \text{ arc length}), \quad (13)$$

is the differential equation of the geodesics based on the differential metric (9) of constant curvature k . This is clear from (11). Let the initial conditions for (13) be

$$y(0) = A \text{ and } \left(\frac{dy(s)}{ds}\right)_{s=0} = B, \quad (14)$$

where A is any element of the abstract space E and B is any element of E such that $[B, g(A, B)] = 1$. By a simple variant of an existence and uniqueness theorem of Hyers and Michal,⁶ the following unique finite equations of the geodesics that satisfy the initial conditions (14) are obtained.

$k > 0$:

$$y(s) = \cos(\sqrt{k}s)A + \frac{1}{\sqrt{k}} \sin(\sqrt{k}s)B. \quad (15)$$

$k < 0$:

$$y(s) = \cosh(\sqrt{-k}s)A + \frac{1}{\sqrt{-k}} \sinh(\sqrt{-k}s)B. \quad (16)$$

$k = 0$:

$$y(s) = sB + A. \quad (17)$$

A number of results now follow readily as in the classical finite dimensional cases. For example—to mention a few—geodesics are closed curves with length $2\pi/\sqrt{k}$ for the case $k > 0$. Hence $2\pi R$ is the length of geo-

desics on a hypersphere (4) in E_c . The "straight line" distance d in the enveloping E_c of two points of the hypersphere (4) in E_c at a geodesic distance s apart is given by

$$d = 2R \sin \left(\frac{s}{2R} \right).$$

5. *Infinite Dimensional Hermitian Differential Metrics.*—In 1935 and again in 1938 the author² had observed that some portions of the theory of finite⁷ dimensional Hermitian differential metrics⁸—and those of constant curvature⁹ in particular—could be generalized to an infinite dimensional theory or to a dimensionless theory. Recently Hua,¹⁰ Siegel¹¹ and others have studied finite dimensional hyperbolic Hermitian differential metrics in matrices. Bochner¹² has conjectured recently that the metrics of Hua and Siegel could be differentially and isometrically imbedded in a fixed elliptic Hermitian differential metric with a countable infinity of dimensions while a construction of Bergmann¹³ can be interpreted as a differential and isometric imbedding into a generalized Fubini metric with a countable infinity of dimensions. Bochner's¹² infinite dimensional differential metrics are special cases of those known to the author.² We intend to say a good deal about this and other related subjects in another paper.

We wish to point out here that if E is a complex abstract Euclidean space³ with $[x, y]$, the independently postulated Hermitian metric, then the following differential metric is Hermitian and has constant real curvature k :

$$ds^2 = [dz, g(z, dz)], \quad (18)$$

where

$$g(z, \xi) = \frac{2}{n^2} \left(u\xi - \frac{k}{2} [\xi, z]z \right), \quad u = 1 + \frac{k}{2} [z, z]. \quad (19)$$

If $[x, y]$ is positive definite and $k \geq 0$, then the ds^2 in (18) is positive definite. Bochner's generalized Fubini metric is an instance of such an abstract metric. The study of various types of subspaces of matrices of (18) would be interesting—especially if E does not have a finite basis.

¹ Michal, A. D., *Bull. Am. Math. Soc.*, **45**, 529–563 (1939), especially pp. 556–559.

² Michal, A. D., these PROCEEDINGS, **21**, 526–529 (1935); *Bull. Am. Math. Soc.* **45**, 529–563 (1939).

³ Michal, A. D., Highborg, I., and Taylor, A. E., *Annali di Pisa*, **6**, 117–148 (1937).

⁴ Michal, A. D., *Bull. Am. Math. Soc.*, **45**, 529–563 (1939), especially Theorem 27.1.

⁵ See, for example, A. D. Michal, *Ibid.*, **45**, 529–563 (1939), especially pp. 552–555. See also A. D. Michal, these PROCEEDINGS, **21**, 526–529 (1935).

⁶ Michal, A. D., and Hyers, D. H., *Annali di Pisa*, **7**, 157–176 (1938), especially Theorem 1.1.

⁷ For references to the work of Poincaré, Picard, Fubini, Study, Cartan and others,

see, for example, Georges Giraud, *Leçons sur les Fonctions Automorphes* (1920), and Elie Cartan, *La Géométrie Projective Complexe* (1931).

⁸ See, for example, D. J. Struik's bibliographical report, *Theory of Linear Connections* (1934), where references are given to the work of Schouten, Van Dantzig, Veblen, Cartan and Kahler. See also the very interesting recent work of S. Chern, *Annals of Math.*, **47**, 85-121 (1946).

⁹ Schouten, J. A., and Van Dantzig, D., *Akad. Wetensch. Amsterdam*, **34**, 1293-1304 (1931).

¹⁰ See, for example, L. K. Hua, *Am. J. Math.*, **66**, 470-488, 531-563 (1944), and *Annals of Math.*, **47**, 167-191 (1946).

¹¹ See, for example, C. L. Siegel, *Am. J. Math.*, **65**, 1-86 (1943) and a few subsequent papers in the *Annals of Math.* See also C. L. Siegel, *Annals of Math.*, **43**, 613-616 (1942).

¹² Bochner, Salomon, *Bull. Am. Math. Soc.*, **53**, 179-195 (1947), especially p. 193.

¹³ Bergmann, S., *J. Reine Angew. Math.*, **169**, 1-42 (1933).

¹⁴ Michal, A. D., *Infinite Dimensional Hypersurfaces of Revolution* (to be published). We thus have numerous examples of infinite dimensional Riemannian spaces with variable curvature and with a non-trivial group of motions.

¹⁵ A metric for the corresponding abstract elliptic case is given by $ds^2 = |dz, g(z, dz)|$, where

$$g(z, \xi) = \frac{(1 + K|z, z|)\xi - K|z, \xi|z}{(1 + K|z, z|)^2}.$$

There is a similar differential metric for an abstract hyperbolic metric differentially imbedded in an $(\infty + 1)$ -dimension "special relativity" metric.

¹⁶ For the definitions and formulas of the classical finite dimensional Riemannian spaces see L. P. Eisenhart, *Riemannian Geometry* (1926); T. Y. Thomas, *Differential Invariants of Generalized Spaces* (1934); Hermann Weyl, *Commentary on Riemann's Über die Hypothesen, welche der Geometrie zu Grunde Liegen* (1923); A. D. Michal, *Matrix and Tensor Calculus with Applications to Mechanics, Elasticity and Aeronautics* (1947).

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*SOME REMARKS ON THE WEIGHTS OF UNKNOWNNS AS
DETERMINED BY THE METHOD OF DIFFERENTIAL
CORRECTIONS*

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In many problems of practical astronomy we apply the method of least square to non-linear equations. The computation is usually done by the method of differential corrections. If the equations of condition are

$$f_i(x, y, \dots) = L_i, \quad i/1 \dots n, \quad (1)$$

where $x, y \dots$ are the unknown parameters to be determined and L_i are the observed quantities, the procedure is the following. We start with certain approximate values for x, y —let us denote them by x_0, y_0 . For simplicity we limit our discussion to the case of two unknown parameters. We determine the corrections Δx and Δy by the least squares method from the equations

$$\frac{\partial f_i}{\partial x} \Delta x + \frac{\partial f_i}{\partial y} \Delta y = L_i - f_i, \quad (2)$$

with x and y in $f_i, \partial f_i/\partial x, \partial f_i/\partial y$ equal to x_0, y_0 . We add Δx and Δy to x_0 and y_0 and, in theory at least, we repeat this procedure until the resulting corrections become equal zero. We shall assume in what follows that this can be achieved. In other words, we make the assumption that the whole procedure is convergent. It is easily seen that the values of x and y which we obtain in this way satisfy the equations

$$\begin{aligned} \sum_{i/1}^n (L_i - f_i) \frac{\partial f_i}{\partial x} &= 0 \\ \sum_{i/1}^n (L_i - f_i) \frac{\partial f_i}{\partial y} &= 0. \end{aligned} \quad (3)$$

Equations (3) are, in fact, nothing but the necessary conditions making the sum of the squares of the residuals a minimum.

When solving (2) by the method of least squares we get the mean errors of the corrections Δx and Δy . These errors are characteristic for Δx and Δy only in so far as we regard Δx and Δy as defined by the normal equations pertaining to equations (2). In almost all cases known to me it is assumed (at least tacitly) that these errors represent also the mean errors of the solution obtained for x and y . It can be easily shown that, in general, this assumption is not valid.*

The inverse weights of Δx and Δy are equal to

$$\sum_{j=1}^n \left(\frac{\partial \Delta x}{\partial L_j} \right)^2 \quad \text{and} \quad \sum_{j=1}^n \left(\frac{\partial \Delta y}{\partial L_j} \right)^2,$$

respectively. We determine $\partial \Delta x / \partial L_j$ and $\partial \Delta y / \partial L_j$ by differentiating with respect to L_j the normal equations pertaining to equations (2). We get (the square brackets are used to denote the summation over i from 1 to n)

$$\left. \begin{aligned} \left[\frac{\partial f}{\partial x} \frac{\partial f}{\partial x} \right] \frac{\partial \Delta x}{\partial L_j} + \left[\frac{\partial f}{\partial x} \frac{\partial f}{\partial y} \right] \frac{\partial \Delta y}{\partial L_j} &= \frac{\partial f_j}{\partial x}, \\ \left[\frac{\partial f}{\partial x} \frac{\partial f}{\partial y} \right] \frac{\partial \Delta x}{\partial L_j} + \left[\frac{\partial f}{\partial y} \frac{\partial f}{\partial y} \right] \frac{\partial \Delta y}{\partial L_j} &= \frac{\partial f_j}{\partial y}. \end{aligned} \right\} \quad (4)$$

By a well-known theorem¹ we find the inverse weights as the diagonal elements of the inverse of the matrix of the coefficients of equations (4).

In order to find the quantities $\partial x / \partial L_j$ and $\partial y / \partial L_j$ that are prerequisite for the determination of the weights of x and y we differentiate equations (3) with respect to L_j and we get

$$\left. \begin{aligned} \left\{ \left[\frac{\partial^2 f}{\partial x^2} (f - L) \right] + \left[\frac{\partial f}{\partial x} \frac{\partial f}{\partial x} \right] \right\} \frac{\partial x}{\partial L_j} + \left\{ \left[\frac{\partial^2 f}{\partial x \partial y} (f - L) \right] + \right. \\ \left. \left[\frac{\partial f}{\partial x} \frac{\partial f}{\partial y} \right] \right\} \frac{\partial y}{\partial L_j} = \frac{\partial f_j}{\partial x}, \\ \left\{ \left[\frac{\partial^2 f}{\partial x \partial y} (f - L) \right] + \left[\frac{\partial f}{\partial x} \frac{\partial f}{\partial y} \right] \right\} \frac{\partial x}{\partial L_j} + \left\{ \left[\frac{\partial^2 f}{\partial y^2} (f - L) \right] + \right. \\ \left. \left[\frac{\partial f}{\partial y} \frac{\partial f}{\partial y} \right] \right\} \frac{\partial y}{\partial L_j} = \frac{\partial f_j}{\partial y}. \end{aligned} \right\} \quad (5)$$

Now, by comparing (4) and (5) we see that the coefficients of the unknowns are not identical in both systems. They are identical when $[(\partial^2 f / \partial x^2)(f - L)]$, $[(\partial^2 f / \partial x \partial y)(f - L)]$, $[(\partial^2 f / \partial y^2)(f - L)]$ equal zero. But these conditions are not fulfilled in general. Even when the residuals $f_i - L_i$ are small and do not show any systematic behavior the considered terms

may be, in cases, quite appreciable. If we have used equations (4) for the determination of the mean errors of Δx and Δy , we should check whether the sums $[(\partial^2 f / \partial x^2)(f - L)]$, $[(\partial^2 f / \partial x \partial y)(f - L)]$, ... are really negligible before we ascribe these errors to the unknowns themselves.[†] That this precaution is essential, we can see from the following numerical example.

Let us suppose that for 5 equidistant values of t : 0, 1, 2, 3, 4, we have observed the values of L , which is supposed to be given by the expression

$$tx + (t - 1)^2y - (t - 1)(t - 3)xy = L. \quad (6)$$

Let the observed values be: -8.0, +4.0, +9.5, +13.5, +11.0. After some trials, we find by the method of differential correction that the solution is: $x = 3$, $y = 1$. The residuals are: 0.0, +1.0, -0.5, +0.5, -1.0. The matrix of the coefficients of the system (4) is

$$\begin{pmatrix} 29, & 48 \\ 48, & 96 \end{pmatrix}.$$

Its inverse is

$$\begin{pmatrix} 0.2, & -0.1 \\ -0.1, & 0.0604 \end{pmatrix}.$$

Hence, denoting by $p_{\Delta x}$ the weight of Δx , by $p_{\Delta y}$ the weight of Δy (with $x = 3$, $y = 1$, both Δx and Δy are equal 0) we obtain

$$p_{\Delta x}^{-1} = 0.2; \quad p_{\Delta y}^{-1} = 0.0604. \quad (7)$$

If we would ascribe these weights to x and y themselves (as is generally done), we would run into a contradiction. We can write (6) in the form

$$A + Bt + Ct^2 = L, \quad (8)$$

with

$$A = y(1 - 3x), \quad B = x - 2y + 4xy, \quad C = y(1 - x). \quad (9)$$

Eliminating x and y between the three equations (9), we get

$$A - C - AB + 3CB + 2AC - A^2 + 3C^2 = 0. \quad (10)$$

Now we can treat A , B , C , as auxiliary (unknown) parameters of the problem and determine them by the least squares method from equations (8) linear in A , B , C , ($t = 0, 1, \dots$; $L = -8.0, +4.0, \dots$) with the condition (10) to be satisfied exactly. It may be done by a standard method² and we get (returning from A , B , C , to x and y), $x = 3$, $y = 1$, the same values obtained by the method of differential corrections. The weights of x and y , p_x , p_y are, however

$$p_x^{-1} = 0.0916; \quad p_y^{-1} = 0.0277, \quad (11)$$

more than two times greater than those given by (7). The discrepancy is of course spurious. If we determine $\partial x/\partial L_j$, $\partial y/\partial L_j$ from equations (5) and form the sums of their squares we obtain exactly the values given by (11).

Summary.—If one uses the method of differential corrections in a least squares solution, the mean errors of the differential corrections to the unknowns are equal to the mean errors of the unknowns themselves only in the special case when the sums of the products of the residuals by the second order partial derivatives of the functions figuring in the equations of the problem are negligible. This is so regardless of how small the differential corrections happen to be. If the sums are not negligible, the equations of the form (5) should be used when determining the weights of the unknowns.

* An extensive discussion of this and related problems is to be found in a paper by E. B. Wilson and R. R. Puffer, "Least Squares and Laws of Population Growth," *Proc. Amer. Acad. Arts Sci.*, **68**, No. 9 (1933). Cf., in particular, equations (25) and (26) and the considerations in the Appendix.

† Evidently the functions of the type $f(x, y, t)$ which fulfill the system of equations

$$\begin{aligned}\partial^2 f / \partial x^2 &= a(x, y) (\partial f / \partial x) + b(x, y) (\partial f / \partial y), \\ \partial^2 f / \partial x \partial y &= a'(x, y) (\partial f / \partial x) + b'(x, y) (\partial f / \partial y), \\ \partial^2 f / \partial y^2 &= a''(x, y) (\partial f / \partial x) + b''(x, y) (\partial f / \partial y),\end{aligned}$$

where $a, b; a', b'; a'', b''$ are arbitrary functions of x and y , independent of t , will have the property of yielding in the standard least squares solution for differential corrections the correct values of the mean errors (t is used instead of subscript i).

¹ Whittaker, E. T., and Robinson, G., *The Calculus of Observations*, London, 1932, p. 241.

² *Ibid.*, p. 252.

*GALACTIC AND EXTRAGALACTIC STUDIES, XVIII. THE
PULSE INDEX FOR EIGHTY-NINE VARIABLE STARS IN THE
MAGELLANIC CLOUDS*

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The Large and Small Clouds of Magellan, because of their isolation from the confusion of Milky Way phenomena, and their distances of only 25 kiloparsecs¹ are ideally situated for the study of the various characteristics of Cepheid variable stars. A fair sampling of the variable star population, suitable for detailed studies, can be taken from the more than 2500 variable stars in the two Clouds. It is thus possible in selecting a representative sample to choose stars with almost any required period-length and avoid those that may be photometrically complicated by companion stars or by dark or bright nebulosity.

The correlations with each other of periods, amplitudes, absolute luminosities and light-curve forms are more satisfactorily examined in the Clouds than in any other region of the sky. The advantages include the negligibility of the distance differentials for the various Cepheids, the apparent brightness of the variables compared with those in other external systems, and the possibility of using for each Cloud a single set of magnitude standards. The light curves can therefore be obtained on a homogeneous basis, both as to the zero point and the magnitude scale.

In an earlier paper of this series² a report was made on 40 variables of the Large Magellanic Cloud. It was shown that the amplitude, with numerous exceptions, varied with period-length; that the form of the light curve was not closely dependent on period (Hertzsprung Effect) except in the vicinity of periods of ten days; and that the period-luminosity relation appeared as usual, as did also the genuine scatter of values, about the mean period-luminosity curve.

A study of a comparable selection of Cepheid variables in the Small Cloud has been undertaken to reinforce, with somewhat richer observational material, the earlier data on the general characteristics of Cepheid light curves, and to take advantage of the still better conditions in the compact Small Cloud for homogeneous photometry.

The observations, the light curves, and a full discussion will be published elsewhere. In this communication a report is made on the asymmetry of the light curves from both Clouds. These curves are of course indicators of the nature of the underlying periodic pulsations.

The prevalent asymmetry in Cepheid light curves can be, and for the Cepheids of the galactic system have been, variously described quanti-

tatively, most commonly in terms of time proportions; for example, by the ratio

$$(t \text{ max.} - t \text{ min.})/\text{period.}$$

S. Gaposchkin³ and others have used the ratios of areas or their differences under specific sections of the light curves as criteria of the degree of asymmetry. C. P. Gaposchkin recently has applied harmonic analysis to a number of Cepheid light curves.⁴

The time of maximum of a Cepheid of short period can be determined with fair accuracy, but the variations at minimum are so slow that even for good light curves the instant of minimum, or the first beginning of the upswing in light, cannot be accurately recorded. The middle of the rise to maximum, however, can be the most accurately observed part of the light curve. A single simple parameter that defines accurately this crucial phase of the pulsation can be obtained from the steepness of the rise; and this pulse-index, if suitably derived, is essentially independent of amplitude of variation and thus is not vitiated by unrecognized optical doubling or by absorption or background effects.

For the derivation of the pulse-index from the light curve, we avoid the slow magnitude variation at minimum, and irregularities like double or hesitant maxima, by defining the relation as follows:

$$\text{p.i.} = \frac{P}{t_{M'} - t_{m'}}.$$

where the period, P , and the times, t , are expressed in the same unit (in days; or better, in period lengths, in which case the numerator is unity), and where M' is the photographic magnitude two tenths of the amplitude fainter than maximum, and m' is the photographic magnitude two tenths of the amplitude brighter than the minimum. (Relative steepness increases with p.i.) By involving only the steepest six tenths of the rise from minimum to maximum in the determination of the index, we avoid completely the uncertainties of curve drawing and the minor fluctuations at maxima and minima.

Various other indices could be used, such as the magnitude change per hour at median magnitude on the rising branch; or the magnitude change per specified fraction of the period; or the ratio of the pulse index as defined above to a comparable index for the descending branch; or smaller fractions of the amplitude might underlie the measures of the pulse index; or intensities might be substituted for magnitudes. No clear advantage, however, would come from these alternatives. In particular, the irregularities that are frequent on the descending branch of a typical Cepheid light curve argue against the use of the descending branch for a quanti-

tative index. Moreover, it can be shown, as in figure 1, that the "recovery-index" derived from the descending branch— $r.i. = P/(t_m - t_M)$ —has a small spread and in consequence is not a sensitive parameter for describing a light curve. Consequently the ratio of p.i. to r.i. has no advantages over the pulse index. In figure 1, lower array, the outstanding r.i. at 9.5 refers to a variable with double maximum, a steep decline, and a period of 9.4 days. In the upper array, which shows the wide spread of p.i., the double maxima light curves are indicated by arrows. The difference in the distribution of p.i. values for the two Clouds is probably not significant. It is most noticeable around $p.i. = 6$.

In table 1 the pulse index and other data are given for both Clouds, the variables arranged in order of increasing period. The Small Cloud stars are marked with asterisks.

In figure 2 the pulse index is plotted against the logarithm of the period, with dots for the Small Cloud, open circles for the Large and crosses showing the means of ten points. The computed correlation coefficient is

$$r(p.i., \log P) = 0.44,$$

and for absolute magnitude,

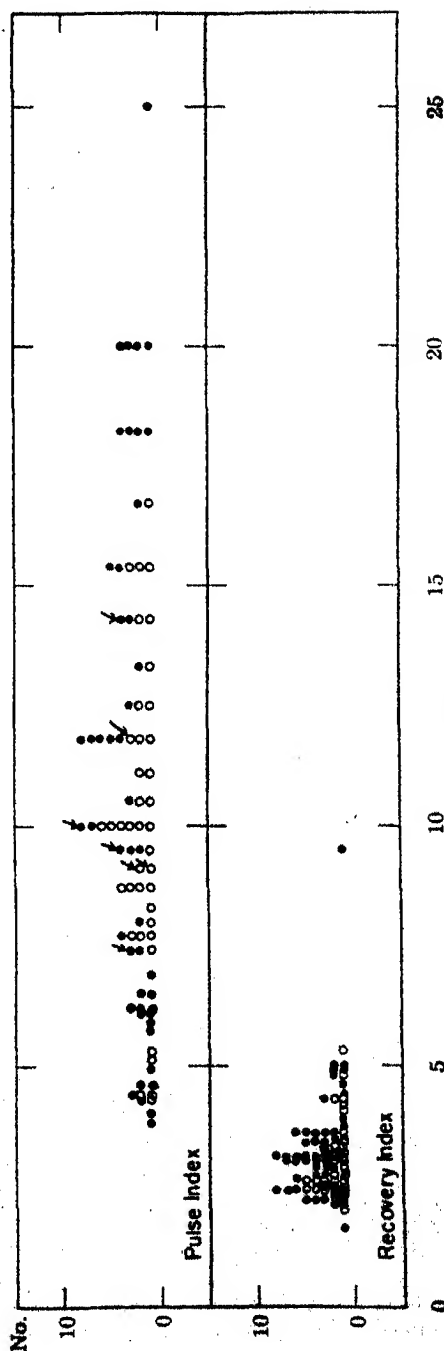


FIGURE 1
Asymmetry in light curves of 89 Cepheid Variables. Open circles, Large Magellanic Cloud; dots, Small Magellanic Cloud.

TABLE I

HARVARD VARIABLE NO.	LOG OF PERIOD	MEDIAN APPARENT MAGNITUDE	AMPLI- TUDE	PULSE INDEX	HARVARD VARIABLE NO.	LOG OF PERIOD	MEDIAN APPARENT MAGNITUDE	AMPLI- TUDE	PULSE INDEX
1505*	0.097	16.42†	0.81	15.4	2752	0.857	16.41	1.26	8.7
1513*	0.134	16.55†	0.60	6.1	927	0.877	15.47	1.20	7.7
10364*	0.177	16.57†	0.96	25.0	2081*	0.881	15.10†	0.74	6.9
2127*	0.263	16.10†	0.61	8.0	816*	0.900	15.61†	0.80	7.4
1446*	0.272	16.62†	1.25	18.2	2722	0.905	15.10	1.26	8.3
2128*	0.356	16.01†	1.06	18.2	1338*	0.929	15.21†	1.24	11.8
2076*	0.398	16.12	1.24	20.0	1790*	0.948	15.28	0.88	9.5
2809	0.399	15.94	1.17	15.4	2103*	0.953	15.49†	0.54	9.5
2344	0.402	16.29	0.66	8.7	971	0.968	15.11	1.10	10.0
2796	0.461	16.26	0.93	10.0	836*	0.973	15.22†	1.05	14.3
1460*	0.464	15.98†	1.24	18.2	1334*	0.975	15.10†	1.26	10.0
2053*	0.508	16.16†	0.83	20.0	952	0.981	15.48	1.07	9.1
2308	0.508	16.36	0.89	12.5	1768*	0.992	15.33	0.43	6.2
2361	0.512	16.51	0.95	13.3	2060*	1.008	14.83	0.96	7.4
11206*	0.531	16.07†	0.68	15.4	818*	1.014	15.15†	1.26	11.8
2472	0.557	16.10	0.78	9.5	1426*	1.019	15.45	0.99	4.9
2085*	0.570	16.29	0.94	13.3	2432	1.038	14.66	0.62	9.1
858*	0.583	15.82†	1.21	20.0	2063*	1.048	15.16†	0.90	4.4
2795	0.593	16.03	1.10	16.7	999	1.051	14.86	0.59	11.8
2788	0.639	15.67	1.05	10.5	2017*	1.057	14.99†	1.12	4.0
1619*	0.641	15.98†	0.83	14.3	2787	1.058	14.99	1.03	4.3
1425*	0.658	15.90†	1.21	11.8	1610*	1.066	15.24†	1.21	3.8
851*	0.671	15.83†	1.19	18.2	905	1.074	14.96	1.40	4.4
2334	0.671	16.21	0.91	7.7	857*	1.079	14.77†	1.22	6.2
2826	0.673	15.83	0.89	15.4	856*	1.085	15.28†	1.12	16.7
951	0.688	16.16	1.01	12.5	1365*	1.094	15.18†	1.11	6.1
2861	0.694	16.00	0.99	8.7	2052*	1.099	14.55†	1.12	4.3
2031*	0.720	15.60	1.29	12.5	1351*	1.117	15.07	0.94	5.9
1818*	0.737	16.16	0.67	7.7	827*	1.129	14.80†	1.07	4.2
815*	0.763	15.89†	0.75	9.5	2463	1.145	14.60	1.61	4.4
2727	0.775	15.55	1.21	10.5	1006	1.153	15.00	1.50	5.1
2619	0.777	15.41	1.38	10.0	1335*	1.158	14.98†	1.23	4.6
2124*	0.783	15.72†	0.82	10.0	933	1.192	15.40	1.49	14.3
5954	0.788	15.79	0.56	8.0	1328*	1.200	14.46	1.27	4.6
1492*	0.799	15.42†	1.19	11.8	1333*	1.212	15.10†	1.37	6.5
2773	0.803	15.55	1.05	8.7	1342*	1.254	14.34†	0.67	5.7
2536	0.804	15.17	1.31	7.4	1005	1.272	14.69	1.46	15.4
2685	0.816	15.40	1.08	7.7	817*	1.276	14.01†	1.16	6.2
2790	0.820	16.01	0.90	11.8	1003	1.387	13.83	1.22	14.3
1400*	0.823	15.64†	0.82	6.5	934	1.450	14.75	1.50	11.1
2358	0.825	15.67	0.70	10.0	823*	1.504	14.44†	1.20	20.0
1855*	0.835	15.89†	0.76	11.8	855*	1.518	14.54†	1.40	10.5
2337	0.837	15.44	1.17	11.8	953	1.680	13.18	1.37	10.0
935	0.849	15.23	1.19	10.0	2447	2.074	13.16	1.01	5.3
2491	0.853	15.36	1.12	11.1					

* Variable in Small Magellanic Cloud.

† Magnitudes by A. S. Carlson; others by V. McK. Nail.

$$r(\text{p.i.}, M) = 0.41.$$

The near equality of these computed coefficients is to be expected since the period-luminosity relation for these 89 stars gives the well-known highly significant correlation,

$$r(M, \log P) = 0.91.$$

The scatter of points in figure 2 shows for this homogeneous material the strength and weakness of the so-called Hertzsprung effect. From the plot, we would say that from periods of five days to periods of fifteen days the steepness tends to decrease measurably. There are, however, several non-conforming stars with periods between eight and twelve days. This is also the interval where double maxima appear, but the double-maxima stars (indicated by arrows) show no trend in steepness with period.

A plot of pulse index against photographic amplitude shows no interdependence of these quantities; the correlation coefficient,

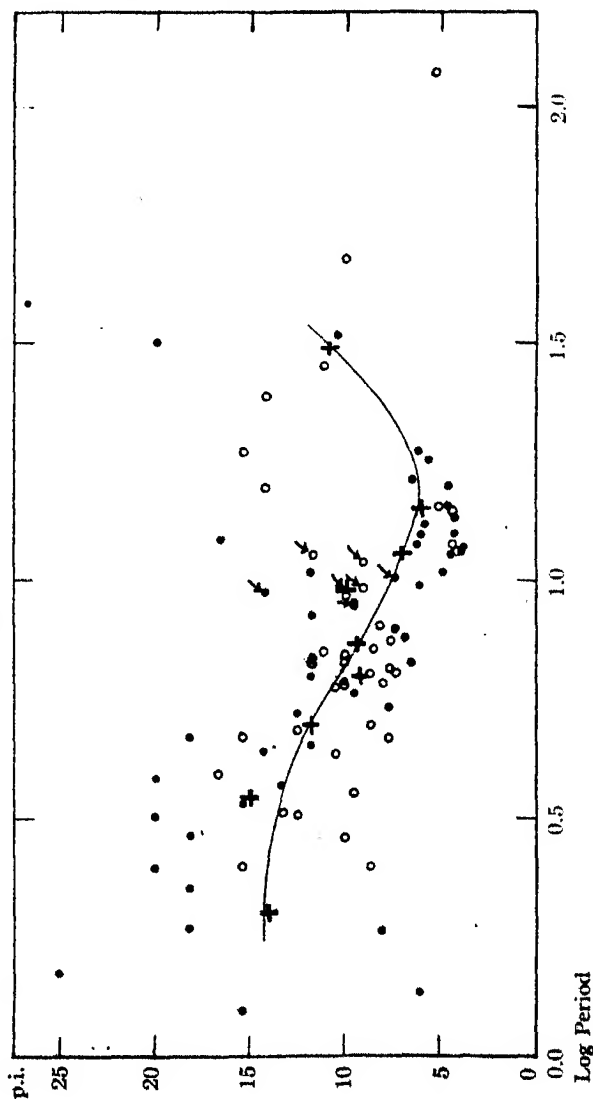


FIGURE 2
The relation of pulse index (ordinates) to logarithm of period.

$$r \text{ (p.i., amp.)} = 0.02,$$

is in agreement with that conclusion.

¹ Current estimates are 23 and 26 kpc, respectively.

² Shapley, McKibben and Mohr, these PROCEEDINGS, 26, 326 (1940).

³ Gaposchkin, S., *Ibid.*, 24, 1 (1938).

⁴ Gaposchkin, C. P., *Abst. Jour.*, 52, 218-226 (1947).

THE *p*-AMINO BENZOIC ACID REQUIREMENT OF THE "SULFONAMIDE-REQUIRING" MUTANT STRAIN OF *NEUROSPORA**

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Emerson and Cushing¹ isolated a strain of *Neurospora* which instead of being inhibited in growth by sulfonamides requires the drug for growth at high temperatures. It seemed as if the roles of *p*-aminobenzoic acid (*PABA*) and sulfanilamide (*SA*) were reversed,² the mold being poisoned by *PABA* and this action being antagonized by *SA*. The evidence suggested that *SA* took the place of *PABA* as a metabolite.

In experiments to test the growth-promoting activity of folic acid compounds for certain *Neurospora* mutants, we found that the sulfonamide-requiring (*sfo*) strain can grow in the complete absence of *SA*. The following different strains³ were used: wild type, *p*-aminobenzoicless (*pab*) of Tatum and Beadle,⁴ sulfonamide-requiring (*sfo*) and the double mutant *p*-aminobenzoicless, sulfonamide-requiring (*pab, sfo*). The results are summarized in table 1.

Pteroyl-tri-glutamic acid has no growth-promoting action at all. Pteroyl-glutamic acid and *p*-aminobenzoylglutamic acid show growth activity for the *pab* mutant. Their activity is about 1 to 2% of the *PABA* activity. Pteric acid seems to be more active, having about 5 to 10% of the activity of *PABA*. At higher concentrations these three substances antagonize *SA* inhibition in a competitive manner. Pteridine was also found to be without activity.

The response of the double mutant is unusual. It requires normally both *PABA* and *SA* for growth. We found that it grows in the absence of *SA* when pteroylglutamic acid or *p*-aminobenzoylglutamic acid is supplied, only slightly when pteric acid is supplied. In the presence of 10^{-4} *M* *SA* the growth is poor with the first two substances, but good with the third one. In the presence of 10^{-7} *M* *PABA* there is no growth with

TABLE 1

GROWTH OF DIFFERENT STRAINS OF *Neurospora* ON FOLIC ACID AND RELATED COMPOUNDS; +++ GOOD GROWTH, ++ FAIR GROWTH, + POOR GROWTH, - NO GROWTH

STRAIN	MEDIUM	COMPOUNDS ADDED ($10^{-4}M$)					
		NONE	PTEROYL- TRIGLUTAMIC ACID	PTEROYL- GLUTAMIC ACID	PTEROIC ACID	p-AMINO- BENZOYL- GLUTAMIC ACID	p-AMINO- BENZOIC ACID
Wild type	Minimal	+++	+++	+++	+++	+++	+++
	$10^{-2} M SA$	-	-	-	-	-	++
<i>pab</i>	Minimal	-	-	++	+++	+++	+++
<i>pab, sfo</i>	Minimal	-	-	++	+	++	-
	$10^{-4} M SA$	-	-	+	+++	++	+++
	$10^{-7} M PABA$	-	-	-	-	-	-
<i>sfo</i>	Minimal	-	-	-	-	-	-
	$10^{-4} M SA$	+++	+++	+++	+++	+++	++

any of the substances tested. The simple sulfonamide-requiring mutant does not grow on any of the folic acid compounds.

The action of all three folic acid compounds can be explained by their free arylamine content. Crystalline folic acid (pteroylglutamic acid) contains about 0.5 to 1% *PABA*, *p*-aminobenzoylglutamic acid about 0.1% and pteric acid about 6%.^{5,6} The *PABA* activity of these compounds is in some instances somewhat higher than can be accounted for by the free arylamine content; it seems likely that a certain amount of cleavage to *PABA* occurs during autoclaving or during growth of the mold. From their inability to substitute for *PABA*, it may be concluded either that folic acid and its derivatives are not products of *PABA* metabolism in *Neurospora*, or else that they have failed to penetrate into the cell in these experiments.

As for the double mutant, we assumed that it grows only at very low *PABA* concentrations, such as those provided as impurities in folic acid. This hypothesis was confirmed by growing the double mutant at low *PABA* concentrations. It can grow well in a very narrow range of *PABA* concentrations (Fig. 1). If we compare the growth rate of the double mutant with that of *pab*, we see that from the lowest *PABA* concentration up both curves ascend at exactly the same slope to about 2×10^{-8} to $5 \times 10^{-8} M$, where they reach the optimum growth velocity. The curve for the double mutant drops rapidly above $5 \times 10^{-8} M$ to show a typically *SA*-requiring growth at *PABA* concentrations higher than $2 \times 10^{-7} M$. The growth between 5×10^{-8} and $2 \times 10^{-7} M$ is typically adaptive: the velocity improves with time, reaching the normal rate at the end. At higher concentrations of *PABA* the curve is very irregular, owing to differences in the degree of adaptation to the absence of *SA*, and to "reversions" caused by mutation.⁷

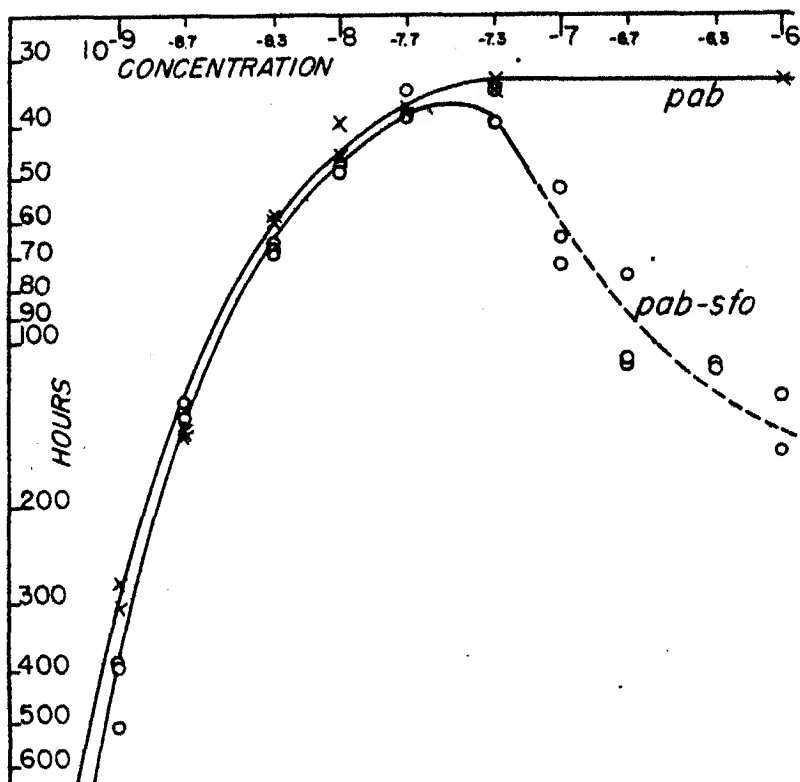


FIGURE 1

Growth of the *p*-aminobenzoicless mutant (-x-x-) and the double mutant *p*-aminobenzoicless, sulfonamide-requiring (-o-o-) on different concentrations of *p*-aminobenzoic acid (experiments Z — 90, Z — 101). Abscissa: concentration of *PABA* in moles; ordinata: time necessary for mycelium to reach 150 mm. in growth tubes.

Looking back to the action of the folic acid compounds, the observed results can be completely explained by the amounts of free *PABA* they contain. These amounts, as deduced from growth of the double mutant, are in agreement with the values obtained by assay with the *p*-aminobenzoicless mutant.

There is only one simple explanation of these facts: in the presence of the gene conditioning the *SA* requirement, *PABA* is poisonous in concentrations which are harmless to the wild type. As the inhibition by *PABA* (at concentrations less than 10^{-3} M) is completely relieved by *SA* in a competitive manner, we can draw a scheme comparable to the scheme of *PABA* action by Kohn and Harris.⁸ They assumed that *PABA* is involved in the synthesis of substances x_1, x_2, \dots, x_n which are necessary

for growth. *SA* competes with *PABA* and inhibits these syntheses. In our case the *sfo* gene would direct a reaction whereby *PABA* produces some inhibitory substance, γ . This reaction must require a somewhat higher *PABA* concentration than is needed for normal growth and therefore is not apparent below a concentration of 10^{-7} *M PABA*. Further, the reaction must be very sensitive to *SA* inhibition and be stopped before *SA* inhibits growth by affecting vital reactions (leading to x_1 , x_2 , etc.). Search is under way for the particular reaction involved.

The sulfonamide-requiring mutant, in the absence of the *pab* gene, obviously produces more *PABA* than corresponds to a concentration of 10^{-7} *M*, so that it poisons itself and requires *SA* for growth as a detoxicant. Hence we have to drop the idea that *SA* is utilized as a metabolite.

Summary.—1. *Neurospora* cannot use pteroylglutamic acid, pterioic acid or *p*-aminobenzoylglutamic acid to replace *p*-aminobenzoic acid.

2. These folic acid compounds contain a certain amount of free *PABA*, which explains a positive growth response of the *p*-aminobenzoicless mutant and the double mutant *p*-aminobenzoicless, sulfonamide-requiring.

3. The double mutant can grow well on low concentrations of *PABA* (10^{-8} to 10^{-7} *M*), but is poisoned by greater concentrations and requires *SA* as a detoxicant.

4. The sulfonamide-requiring strain must produce more than the tolerated amount of *PABA* and thus inhibits itself.

Acknowledgment.—The author wishes to express his indebtedness to Dr. Sterling Emerson for suggesting the problem and for offering advice and help throughout the course of the work.

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¹ Emerson, S., and Cushing, J. E., "Altered Sulfonamide Antagonism in *Neurospora*," *Federation Proc.*, **5**, 379-389 (1946).

² Emerson, S., "Growth Responses of a Sulfonamide-Requiring Mutant Strain of *Neurospora*," *J. Bact.*, **54**, 195-207 (1947).

³ The particular strains used are: wild type E5256A, *pab* 1633 reisolated as Z35-I-8, *sfo* E15172 reisolated as E16014A and *pab*, *sfo* 1633 and E15172 combined in strain E19335.

⁴ Tatum, E. L., and Beadle, G. W., "Genetic Control of Biochemical Reactions in *Neurospora*: An Aminobenzoicless Mutant," *Proc. Nat. Acad. Sci.*, **28**, 234-243 (1942).

⁵ Williams, R. D., "The Comparative Antisulfanilamide Activity of *p*-Aminobenzoyl-1(+)-glutamic Acid and *p*-Aminobenzoic Acid," *J. Biol. Chem.*, **156**, 85-89 (1944).

⁶ Lampen, J. O., and Jones, M. J., "The Antagonism of Sulfonamide Inhibition of Certain Lactobacilli and Enterococci by Pteroylglutamic Acid and Related Compounds," *Ibid.*, **166**, 435-448 (1946).

⁷ Emerson, S., "A Physiological Basis for Some Suppressor Mutations and Possibly for One Gene Heterosis," *Proc. Nat. Acad. Sci.* (in press).

⁸ Kohn, H. I., and Harris, J. S., "On the Mode of Action of Sulfonamides. I. Action on *Escherichia coli*," *J. Pharm. Exp. Therap.*, **73**, 343-361 (1941). Harris, J. S., and

Kohn, H. I., "On the Mode of Action of Sulfonamides. II. The Specific Antagonism Between Methionine and the Sulfonamides in *Escherichia coli*," *Ibid.*, **73**, 383-400 (1941).

DIRECTED FERTILIZATION IN MAIZE

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Ordinarily the two gametes in the maize pollen grain are genetically identical. However, a plant carrying an A-B interchange produces a pollen grain in which the gametes are not alike.¹ This paper deals with the rôle of the dissimilar gametes in fertilization.

An A-B interchange is one between the supernumerary B-type chromosome² and a member of the basic complement (A-type chromosome). The behavior of the interchange chromosomes during the development of the pollen grain may be illustrated by the case of TB-4a. In this interchange, the distal seven-eighths or so of the short arm of chromosome 4 was transferred to a segment of a B-type bearing the centromere of the latter. The resulting chromosome is designated the B⁴ chromosome. The other interchange chromosome (4^B) contains the rest of chromosome 4 and most or all of the distal heterochromatic segment of the B-type.

The 4^B chromosome is orthodox in its behavior and is found regularly in each gamete of the pollen grain. The B⁴ chromosome also follows the normal pattern until the division of the generative nucleus is reached. In this division the B⁴ chromosome frequently undergoes non-disjunction so that one of the gametes receives two B⁴ chromosomes and the other receives none. A second type of pollen grain is the result of normal disjunction; both gametes of the pollen grain are identical, each containing one B⁴ chromosome.

Three classes of seed are expected when a normal seed parent is crossed with a pollen parent homozygous for TB-4a. Two of these are obtained from fertilization involving the first type of pollen grain. If the gamete that is deficient for the B⁴ chromosome fertilizes the egg and its partner unites with the polar nuclei, a seed with a deficient embryo and a hyperploid endosperm will result (Class I). If the gametes exchange their respective rôles in fertilization, the seed will have an embryo hyperploid for the B⁴ chromosome and a deficient endosperm (Class II). A third kind of seed is produced when the second pollen type is involved; in this case, the embryo is heterozygous for the interchange and the endosperm also carries a single B⁴ chromosome (Class III).

The three classes of seed may be readily identified through the use of the sugary endosperm gene, *su*. The B⁴ chromosome carries the dominant allele *Su*. If the seed parent is homozygous for *su*, the three kinds of seed would have, respectively:

Class I: A non-sugary, hyperploid endosperm and a deficient embryo, hemizygous for *su*;

Class II: A sugary, deficient endosperm and an embryo of composition *su Su Su*; and

Class III: A non-sugary, euploid endosperm with a euploid embryo of composition *su Su*.

If fertilization involving the first type of pollen grain occurs in either direction with equal frequency (i.e., random fertilization), seed classes I

TABLE 1
RESULTS OF CROSSES BETWEEN NORMAL SEED PARENTS HOMOZYGOUS FOR *su* AND
POLLEN PARENTS HOMOZYGOUS FOR TB-4a AND *Su*

CROSS	SUGARY SEEDS	TOTAL SEEDS	% SUGARY	*S.E.
GC-1 × 25-2	162	271	59.8	3.0
2 × 25-2	175	267	65.5	2.9
3 × 25-2	141	217	65.0	3.2
4 × 25-2	158	229	69.0	3.1
5 × 25-2	106	218	48.6	3.4
107-3 × 25-2	164	274	59.9	3.0
107-4 × 25-2	130	262	51.9	3.1
2268-3 × 25-2	130	210	61.9	3.4
2269-2 × 25-2	226	410	55.1	2.5
2269-7 × 25-2	314	504	62.3	2.2
108-28 × 50-13	76	132	57.6	4.3
273-2 × 50-13	124	216	57.4	3.4
263-10 × 50-14	169	303	55.8	2.9
277-4 × 50-14	74	118	62.7	4.5
Totals	2155	3631	59.4	0.8

and II should be equal in number assuming they are equally viable. The number of seeds with a sugary endosperm (Class II) would not be expected, on this basis, to exceed the number of non-sugary seeds. Actually, the sugary seeds should be in the minority since the non-sugary seeds are expected to include not only those of Class I but also those of Class III, the latter derived from fertilization involving the second type of pollen grain. The results of crosses published previously¹ gave an average value, of approximately 50% for the proportion of sugary seeds and this was interpreted, assuming random fertilization, as an indication of a very low rate of normal disjunction. There were two crosses, however, in this first

group that gave what appeared to be a significantly higher proportion of sugary seeds. Subsequent crosses involving three different but closely related pollen parents, cytologically identified as homozygous for TB-4a, have given a segregation of sugary seeds clearly in excess of 50% (table 1). It should be noted that these data are not selected but represent the total harvest of crosses involving these parents.

The extent to which the sugary seeds outnumber their reciprocal counterparts (Class I) cannot be determined directly from the data in table 1, since the non-sugary seeds that have a hyperploid endosperm are indistinguishable from those with a euploid endosperm (Class III). The two types of non-sugary seeds may, however, be separated on the basis of the difference between their embryos. This may be accomplished, in the plants grown from these seeds, by a direct cytological examination of the microsporocytes, by an examination of the mature pollen for the percentage of aborted grains, or on the basis of sugary ratios in crosses with a *su su* parent. The identification methods employed will be described in detail elsewhere.

TABLE 2
EMBRYO COMPOSITION OF NON-SUGARY AND SUGARY SEEDS OF CROSSES GIVEN IN TABLE 1. SEE TEXT FOR FURTHER EXPLANATION

CROSS	NON-SUGARY SEEDS			SUGARY SEEDS		
	(CLASS I) —	(CLASS III) +	++	—	+	++ (CLASS II)
GC-4 × 25-2	25	28	1	1	1	131
GC-5 × 25-2	40	32	2	0	0	87
2269-2 × 25-2	44	16	2	0	1	83
2269-7 × 25-2	43	48	0	0	0	81
Totals	152	124	5	1	2	382

Table 2 shows the distribution of embryo types found in non-sugary seeds. Also included for comparison are the types contained in sugary seeds. The symbols —, + and ++ refer, respectively, to: (1) an embryo deficient for the B⁴ chromosome, (2) a heterozygous embryo, carrying one B⁴ chromosome and (3) a hyperploid embryo, with two B⁴ chromosomes. Among the non-sugary seeds, only about half contain the deficient embryo indicative of Class I.

The data in table 2 also reaffirm the conclusion reached from previous evidence that a plant homozygous for TB-4a produces two types of functional pollen grains. With only eight exceptions among 681 seeds tested, the seeds fall into the three classes described above. The exceptions would be expected normally, as a consequence of heterofertilization,⁴ in which the gametes of two different pollen grains unite with the egg and polar nuclei of the same embryo sac.

If we assume from the data in table 2 that half of the non-sugary seeds belong to Class III and apply this correction to the data in table 1, the

preponderance of Class II seeds over Class I becomes even more evident. The cross 2269-7 \times 25-2 gave 314 sugary seeds and 190 non-sugary seeds; when the correction is applied, the ratio of Class II to Class I is 314 to 95, or roughly 3:1. In the cross GC-5 \times 25-2, which gave a relatively low percentage of sugary seeds, the ratio is 106 to 56, or approximately 2:1. If we take 60% as an average value for the frequency of sugary seeds in all of the crosses of table 1, the proportion of Class I:Class II:Class III becomes, using the same correction, 1:3:1. Thus, four-fifths of the progeny were obtained from fertilizations involving pollen grains in which non-disjunction had occurred and in three-fourths of these, the deficient gamete united with the polar nuclei and the hyperploid gamete with the egg.

It was expected, assuming random fertilization, that the Class I and Class II seeds would occur in equal numbers. The low frequency of Class I seed brings up the question of the relative viability of this type. Are these seeds being lost in development? If this were so, it should result in a detectable amount of sterility on the ear since the loss would comprise two-thirds of this class or two-sevenths of the total population. Actually, several of the ears in table 1, including those which gave the most disproportionate ratios, had full sets of seed. It seems likely, therefore, that the low proportion of Class I seeds is evidence that fertilization is not random; that the union of the egg with the hyperploid gamete and the concomitant fusion of the polar nuclei with the deficient gamete is preferred to the reverse order of fertilization, in which the deficient gamete unites with the egg and the hyperploid gamete with the polar nuclei.

Similar results have been obtained with TB-9b, an interchange involving chromosome 9 of the basic set. The break in chromosome 9 occurred about halfway along the short arm, between *C* and *Wx*; the B-type chromosome was broken within the distal heterochromatic segment. In the cross to be described, the B^0 chromosome carried *C* (for anthocyanin-colored endosperm) and 9^B chromosome carried *Wx* (for starchy endosperm). The TB-9b pollen parents used in the cross were homozygous for the 9^B chromosome and hemizygous for the B^0 chromosome (with respect to chromosome 9, their constitution was $9^B 9^B B^0$). Such a plant is equivalent, as regards male transmission, to the homozygote (with two B^0 chromosomes), since only the microspores possessing both a B^0 and a 9^B chromosome develop into functional pollen grains. The microspores that are deficient for the B^0 chromosome produce aborted pollen grains with a distinctly reduced starch content.

The seed parents used in the cross were cytologically normal and homozygous for *c* and *wx*. In the TB-9b pollen parent, the B^0 chromosome undergoes non-disjunction in the division of the generative nucleus. A pollen grain is thus produced in which one gamete has two B^0 chromosomes

and the other has none (and therefore lacks *C*). The union of the hyperploid gamete with the polar nuclei and the deficient gamete with the egg would give a kernel with a hyperploid, colored endosperm and a deficient embryo (analogous to the Class I seed obtained from the crosses involving TB-4a). The reciprocal order of fertilization would produce a seed with a deficient, colorless endosperm and a hyperploid embryo (Class II). The results of six crosses, involving two TB-9b parents are given in table 3. Here, as with TB-4a, the seeds with a deficient endosperm are clearly in the majority. With rare exception, the seeds of both classes were starchy, an indication of the regularity of disjunction of the 9^B chromosome in the division of the generative nucleus.

TABLE 3
SEE TEXT FOR DESCRIPTION OF CROSS

CROSS	SEEDS WITH COLORLESS ENDOSPERM	TOTAL SEEDS	% COLORLESS	±S.E.
119-4 × 96-8	96	148	64.9	3.9
119-11 × 96-8	235	356	66.0	2.5
2264-7 × 96-8	198	334	59.3	2.7
119-5 × 96-15	214	305	70.2	2.6
119-10 × 96-15	150	249	60.2	3.1
2264-11 × 96-15	220	353	62.3	2.6

Scutellum color was used as an indicator of the presence of the B⁹ chromosome in the embryos of the colored and colorless seeds. *C*, in addition to certain other known genes, is required for anthocyanin color in the scutellum.⁴ Two of the crosses gave an appropriate complementary genotype and the seeds obtained from these were classified for scutellum color in relation to endosperm color. It was anticipated that the Class I seeds should have a colorless embryo and the Class II seeds should have a colored embryo. The results given in table 4 show that over 97% of the

TABLE 4
RELATION OF SCUTELLUM COLOR TO ENDOSPERM COLOR IN TWO CROSSES OF TABLE 3

CROSS	COLORED ENDOSPERM*		COLORLESS ENDOSPERM†	
	COLORRED SCUTELLUM	COLORLESS SCUTELLUM	COLORRED SCUTELLUM	COLORLESS SCUTELLUM
119-4 × 96-8	0	52	95	1
119-11 × 96-8	9‡	108	232	2
Totals	9	160	327	3

* Three others lacked an embryo and one other was also unclassifiable for scutellum color.

† One other lacked an embryo.

‡ One had a colorless endosperm sector.

seeds fall into these two categories and that the Class II seed outnumbers its reciprocal counterpart by approximately 2:1.

Twelve other seeds, not of Class I nor II, were also obtained. Three had a colorless endosperm as well as a colorless embryo; they were therefore deficient in both endosperm and embryo for the B^9 chromosome. They may be accounted for as cases of heterofertilization in which deficient gametes from two different pollen grains were involved. The nine seeds with a colored endosperm and a colored embryo may also be the result of heterofertilization, this time involving two hyperploid gametes. Or they may represent fertilization involving a pollen grain in which both gametes contain one B^9 chromosome, as a consequence of normal disjunction of this chromosome (thus equivalent to the Class III seeds of table 2). The frequency of normal disjunction appears to be much lower for the B^9 chromosome than for the B^4 chromosome. Whether this is a characteristic difference between the two chromosomes or is an expression of a difference in genotype in the two parental types is a question for further investigation.

The evidence from the crosses with TB-4a and TB-9b may be summarized as follows. Plants carrying either of these interchanges produce, in the main, one type of pollen grain. This contains one gamete deficient for the aberrant chromosome (B^4 or B^9 , as the case may be) and another gamete carrying the chromosome in duplicate. Fertilization involving the two gametes may proceed in either of two ways: (1) the deficient gamete may unite with the polar nuclei to form the primary endosperm nucleus and the hyperploid gamete fertilizes the egg or (2) the hyperploid gamete may unite with the polar nuclei and the deficient partner with the egg. The first fertilization pattern is much more prevalent than the second. In the case of TB-4a, it was estimated that the first occurred three times as frequently as the second; with TB-9b, it was twice as frequent. The preference of one pattern over the other will be referred to by the term "directed fertilization."

Two possible explanations that will account for directed fertilization are being tested. The first of these assumes a specific orientation of the deficient and the hyperploid gametes within the pollen grain or at some time prior to fertilization. According to this hypothesis one of the gametes would have a positional advantage over the other that would result in the preferential union of the hyperploid gamete with the egg and the deficient gamete with the polar nuclei. The other explanation assumes a physiological difference between the hyperploid and deficient gametes that results in directed fertilization.

The assumption that the gametes are oriented within the pollen grain presupposes that one of the oriented gametes receives both of the non-disjoining chromosomes more often than the other. Evidence for a directed distribution such as this has been demonstrated cytologically in other organisms in which mitotic non-disjunction occurs. In *Sciara co-*

prophila, the sex chromosome moves to a specific pole in the division of the secondary spermatocyte.⁵ In rye, the supernumerary chromosome, which undergoes non-disjunction in the first division of the microspore, is found more often in the generative nucleus than in the vegetative nucleus.⁶ The products of non-disjunction of the supernumerary chromosome in sorghum are directed to the generative pole in the exceptional division of the vegetative nucleus.⁷

It is doubtful that the deficiency or duplication of A-chromatin (the segment of chromosome 4 in the B⁴ chromosome or chromosome 9 in the B⁹ chromosome) in the gametes is an essential factor in determining directed fertilization. An indication of directed fertilization is also found in the results of a cross in which the pollen parent had two intact B-type chromosomes and a normal basic complement. When the seed parent was devoid of B-types, most of the progeny possessed either two B-types or none in their embryos. The progeny with two B-types were in a marked majority.^{2, 8} This may be interpreted as follows: (1) the 2B parent produces a pollen grain in which one gamete has two B-types and the other has none; and (2) the 2B gamete has an advantage over its OB partner in the fertilization of the egg.

Directed fertilization of the egg by the 2B gamete is also of interest in connection with the problem of the survival of the B-type chromosome. This chromosome is subject to fragmentation and also to loss in meiosis.^{2, 9} Yet it persists in a number of maize varieties and is particularly prevalent in certain sugary strains, notably the Black Mexican. The preferential fertilization of the egg by the gamete containing the B-types, after non-disjunction, would provide an excess of B-types to counteract the fragmentation and loss of this chromosome. The common occurrence of the B-type in certain varieties could then be accounted for if the mechanism of directed fertilization is more highly developed in these strains, as compared with others in which the B-type is absent or rarely found.

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† Permanent address. A grant from the Agnes H. Anderson Research Fund for technical assistance is gratefully acknowledged.

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² Randolph, L. F., *Ibid.*, **26**, 608-631 (1941).

³ Sprague, G. F., *Ibid.*, **17**, 358-368 (1932).

⁴ Sprague, G. F., *U. S. Dept. Agr. Tech. Bull.*, **292**, 1-43 (1932).

⁵ Metz, C. W., *Am. Naturalist*, **72**, 485-520 (1938).

⁶ Müntzing, A., *Hereditas*, **32**, 97-119 (1946).

⁷ Darlington, C. D., and Thomas, P. T., *Proc. Roy. Soc. London*, **130**, 127-150 (1941).

⁸ Supplementary data (unpublished) were kindly furnished by Dr. A. E. Longley. Among the examined progeny from seven OB × 2B crosses, 31 had 2B's and 13 had none.

⁹ Darlington, C. D., and Upcott, M. B., *J. Genet.*, **41**, 275-296 (1941).

ABSTRACT ERGODIC THEOREMS*

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1. The mean ergodic theorem of J. von Neumann has been extended by various authors—notably F. Riesz¹ and Yosida and Kakutani²—to an abstract theorem asserting the convergence to a fix point of the means $T_n x = (1/n + 1) \sum_{j=0}^n T^j x$, where T is a linear transformation in a Banach space E . Alaoglu and Birkhoff³ replaced the iterates (T^j) by a semi-group G of linear transformations and showed that the convergence of certain general means of transforms of an element x of E is equivalent to the existence (and uniqueness) of a fix point y in the closed convex hull of the orbit of x under G . In this note we indicate how relaxation of the uniform boundedness and countability restrictions customarily imposed on G leads to a more general theory, which not only embraces a significantly wider domain of phenomena but subsums previous results in a sharper and more transparent form. Details and further developments will appear elsewhere.

2. Consider a Banach space E and a semi-group G of linear transformations T of E into itself. We denote by $G^* = [\sum a_j T_j | a_j \geq 0, \sum a_j = 1, T_j \in G]$ the family of transformations T^* consisting of all finite convex combinations of the elements T of G , by \bar{G}^* the closure of G^* in the uniform topology of operators, by $0(x) = [T^* x | T^* \in G^*]$ the orbit of x under G^* , and by $\bar{0}(x)$ the closure of $0(x)$ in E . G^* and \bar{G}^* are clearly semi-groups of operators, and $0(x)$ and $\bar{0}(x)$ are convex sets. We may and shall assume that G contains the identity transformation.

Definition 1: G is an ergodic semi-group if it possesses at least one system of almost invariant integrals. By such a system we mean a family of transformations (T_α) , indexed by the directed set (α) , with the following properties:

- I. T_α is a linear transformation of E into itself for every α .
- II. For every x and all α $T_\alpha x \in \bar{0}(x)$.
- III. There exists an $M > 0$ such that $\|T_\alpha\| \leq M$ for all α .
- IV. For every x in E and all T in G
 - (a) $\lim_{\alpha} (TT_\alpha x - T_\alpha x) = 0$;
 - (b) $\lim_{\alpha} (T_\alpha T x - T_\alpha x) = 0$.

If the limits IV hold uniformly for all x in any bounded set, we call the system (T_α) a uniform system. IV remains valid if T is replaced by any element U of \bar{G}^* .

3. The following examples provide some indication of the scope of the theory. Obvious generalizations will suggest themselves. It must be emphasized (Theorem 1 infra) that the essential phenomena depend on the existence of *at least one* system (T_α) , rather than on the particular system exhibited.

Example 1: Standard Case. G consists of the iterates (T^n) of a linear transformation T and the T_α are the previously defined T_n under the natural ordering. I and II are trivially satisfied, and the usual condition that $\|T^n\| \leq M$ for all n ensures the validity of III, and of IV in the uniform sense. However, as pointed out in examples by Hille⁴ and Dunford,⁵ uniform boundedness of the T^n is too restrictive: all one needs is a bound on the $\|T_n\|$ and $\lim T^n/n = 0$ in an appropriate sense.

Example 2: Almost Periodic Functions. E is the Banach space of complex-valued bounded continuous functions $f(x)$ ($-\infty < x < \infty$) with $\|f\| = \sup |f(x)|$. G is the group of translations $f(x) \rightarrow f(x+a)$, whose only fix points are the constant functions. One may take as the system (T_α) the Bohr means

$$T_\alpha f = \frac{1}{\alpha} \int_0^\alpha f(x+t) dt \quad (\alpha > 0)$$

under the natural ordering.

Example 3: Bounded Abelian Semi-Group. If G is Abelian and if $\|T\| \leq M$ for all T in G , then the elements of G^* form a uniform system of almost invariant integrals under the ordering: $U \supset V$ if and only if there exists W in G^* such that $U = WV$. (That this ordering has the required composition property follows from the fact that $UV = VU$ is a common successor of U and V .) Clearly this example includes Example 2 and the bounded case of Example 1.

Example 4: Summability of Fourier Series. E is the space C of continuous real-valued functions of period 2π , with $\|f\| = \sup |f(x)|$. G consists of the transformations $U_n f = f - S_n$, where $S_n(x)$ is the sum of the first $2n+1$ terms of the formal trigonometrical series corresponding to f . The identity $U_n U_m = U_v$, where $v = \max(m, n)$, implies that G is an Abelian semi-group, whose only fix point is the null function. The $\|U_n\|$ are unbounded—if bounded the U_n themselves would form a system of almost invariant integrals for G ; and this hypothesis would in turn imply the uniform convergence of the Fourier Series of every continuous function, which is false. However, the $(C, 1)$ means $T_n = (1/n + 1) \sum_0^n U_j$, do form a system of almost invariant integrals, the existence of the bound in III following from the uniform $L_1(-\pi, \pi)$ bound of the Fejér kernel.

The above examples are all of the Abelian type. The more difficult non-commutative case is discussed in § 5.

4. We come now to the first key theorem and the definition of an ergodic element that it inspires.

THEOREM 1. *If G is ergodic, x an element of E , and (T_α) ANY system of almost invariant integrals, then the following conditions on an element y in E are equivalent:*

- (1) $y \in \bar{0}(x)$, and $Ty = y$ for all $T \in G$;
- (2) $y = \lim_{\alpha} T_\alpha x$;
- (3) $y = \lim_{\alpha} T_\alpha x$ weakly;
- (4) y is a weak cluster point of $(T_\alpha x)$.

Definition 2: If G is ergodic, x is ergodic with (unique) limit fix point y if and only if there exists a $y = T_\infty x$ satisfying any of the conditions (1) to (4) above.

THEOREM 2. *If G is ergodic, the ergodic elements of E constitute a closed invariant subspace Γ . The transformation $T_\infty x = y = \lim_{\alpha} T_\alpha x$ is a linear transformation of the space Γ into itself, and $\|T_\infty\|_{\Gamma} \leq M$. Moreover, on Γ we have $T_\infty = T_\infty^2 = T_\infty U = UT_\infty$ for every U in G^* .*

The implications (2) \rightarrow (3) \rightarrow (4) of Theorem 1 are trivial. In verifying the crucial implication (4) \rightarrow (1) we employ the Mazur theorem⁶ to obtain $y \in \bar{0}(x)$ and then show that $f(Ty - y) = 0$ for all T in G and all f in E^* . IV (a) is required only to establish (4) \rightarrow (1), IV (b) only in proving (1) \rightarrow (2).

Condition (4) is the most useful condition for ergodicity. The required y exists automatically when the set $(T_\alpha x)$ or $[Tx | T \in G]$ is (conditionally) weakly compact. From this remark follow the standard mean ergodic theorems (Example 1) and the existence and uniqueness of the mean for generalized almost periodic functions defined on Abelian groups (Examples 2 and 3). Proof of the Fejér theorem (Example 4) proceeds thus: Since $\lim_n T_n f = 0$ in $L_2(-\pi, \pi)$, there exists a subsequence $T_{n_i} f$ converging to 0 almost everywhere. The Lebesgue convergence theorem then implies that $\lim_{n_i} T_{n_i} f = 0$ weakly in C , whence $\lim_n T_n f = 0$ strongly in C .

Conditions on the operators T of G sufficient to ensure ergodicity are of interest. Yosida and Kakutani found the special (bounded) case of Example 1 in which T is (weakly) quasi-completely continuous fundamental in their abstract treatment of Markoff processes.² [A transformation T is called (weakly) 'quasi-completely continuous' if there exists an integer m and a (weakly) completely continuous transformation V such that $\|T^m - V\| < 1$.]

THEOREM 3. *If G is (ergodic) uniformly ergodic and if some T in \bar{G}^* is (weakly) quasi-completely continuous, then every x in E is ergodic. $T_\infty = \lim T_\alpha$ (strongly) uniformly, and the projection T_∞ is (weakly) completely*

continuous. The set $T_\alpha(E)$ of fix points is a (reflexive) finite dimensional subspace of E .

Not only is this theorem a considerable generalization of the ergodic theorems of Yosida and Kakutani, but our proof is much simpler. The principal tool is the identity

$$T_\alpha = (I - D)^{-1}VT_\alpha + (I - D)^{-1}(T_\alpha - T^\infty T_\alpha),$$

where $D = T^\infty - V$.

5. In the general non-Abelian case the existence of a system of almost invariant integrals and the definition itself of an ergodic element are obscure. If the T in G are uniformly bounded, however, the ordering of G^* in Example 3, although no longer of Moore-Smith type, leads naturally to the Alaoglu-Birkhoff definition⁷ of ergodicity:

Definition 3: If G is bounded, an element x of E is ergodic if and only if the means T^*x converge to a (unique fix) point y .

(When G is both bounded and ergodic, definitions 2 and 3 coincide.) The ergodic elements again form a closed invariant subspace Γ . It is clear that a necessary condition for ergodicity of x is that $\bar{O}(x)$ contain a unique fix point; that $\bar{O}(x)$ contain a unique fix point for each element x of an invariant set is a sufficient condition. Algebraic restrictions on the space E or the semi-group G may reduce the problem to establishing only the existence of a fix point y in $\bar{O}(x)$.

THEOREM 4: Let G_1, G_2 be bounded semi-groups of operators such that $UV = VU$ for every U in G_1 and V in G_2 , and let every fix point of G_1 be a fix point of G_2 and conversely. Then

- (1) The fix points of $\bar{O}_1(x)$ and $\bar{O}_2(x)$ reduce to a unique common fix point.
- (2) If both $\bar{O}_1(x)$ and $\bar{O}_2(x)$ contain a fix point y for every x in an invariant set Γ , then every x of Γ is ergodic with respect to G_1, G_2 , and G_1G_2 with the limit fix point y .

When $E = \Gamma$ is the set of complex-valued almost periodic functions—in the sense of von Neumann⁸—defined on an arbitrary group, one takes G_1 as the group of left translations, G_2 as the group of right translations. A combinatorial lemma of Maak⁹ permits the construction of a system (T_α) satisfying I, II, III and IV (a)—sufficient to ensure the existence of a fix point in every $\bar{O}_1(x)$ or $\bar{O}_2(x)$. Theorem 4 then yields the existence of the mean in the form of an ergodic theorem. The existence of the invariant integral mean and Haar measure in compact groups is, of course, a special case. It is characteristic of this approach that the uniqueness theorem becomes an automatic consequence of Theorem 1.

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² Yosida, K., and Kakutani, S., *Ann. Math.*, **42**, 188-228 (1941).

³ Alaoglu, L., and Birkhoff, G., *Ibid.*, **41**, 293-309 (1940).

⁴ Hille, E., *Trans. Am. Math. Soc.*, **57**, 246-269 (1945).

⁵ Dunford, N., *Ibid.*, **54**, 185-217 (1943).

⁶ For the requisite properties of the weak topology see Eberlein, W. F., these *PROCEEDINGS*, **33**, 51-53 (1947).

⁷ For this definition and proofs of some of the immediately following remarks see Alaoglu, L., and Birkhoff, G., *loc. cit.*

⁸ Neumann, J. von, *Trans. Am. Math. Soc.*, **36**, 445-492 (1934).

⁹ Maak, W., *Hamburg Abhandlungen*, **11**, 240-244 (1935).

CLOSED SURFACES WITHOUT CONJUGATE POINTS*

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The present note contains the proof of the following

THEOREM. *Let S be a closed surface of class C''' in the sense of Riemannian geometry. If no geodesically conjugate points exist on S the total curvature of S must be negative or zero. In the latter case the Gaussian curvature must vanish everywhere on S .*

From the second part of the theorem and from known facts one infers: if S is the topological image of the torus or of the Klein bottle and if F contains no geodesically conjugate points S must be the one-to-one and isometric image of a Euclidean model of such a surface.

This second part of the theorem also forms the subject of a recent paper by Morse and Hedlund.¹ These authors prove the theorem under an additional hypothesis about S (non-existence of focal points on S) and raise the question if the theorem holds true without this restriction.

The idea underlying the proof for the first part of the theorem had been outlined in a previous paper by the author.² It was found that only little additional remarks are required to make the proof cover the entire theorem. In the present note the proof will be developed ab ovo.

Proof of the Theorem. Consider the Jacobi differential equation of normal variation

$$y''(s) + K(s)y(s) = 0 \quad (1)$$

(K = curvature) along an arbitrary oriented geodesic on S . The direction of increasing arc length is always chosen as to coincide with the direction of the geodesic. The solutions of (1) are well known to exist on the whole axis of s . Non-existence of conjugate points means: any non-trivial solution of (1) possesses at most one zero, any two non-identical solutions intersect at most once. Let

$$y(s; a, b)$$

be the (according to this intersection property unique) solution satisfying

$$y(a; a, b) = 1, \quad y(b; a, b) = 0. \quad (2)$$

These functions satisfy the identity

$$y(s; a, b) = y(\alpha; a, b)y(s; \alpha, \beta) + y(\beta; a, b)y(s; \beta, \alpha). \quad (3)$$

Both sides represent solutions of (1) which, according to (2), intersect at $s = \alpha, \beta$ and which therefore coincide. In the special case $\alpha = a'$ and $\beta = b$ (3) becomes

$$y(s; a, b) = y(a'; a, b)y(s; a', b). \quad (3')$$

From (2) and from the intersection property we infer that

$$y(s; a, b') > 0 \text{ for } s < b' \text{ and } a < b'. \quad (4)$$

The two solutions $y(s; a, b)$ and $y(s; a, b')$, $b < b'$, intersect at $s = a$ but nowhere else. Hence, on account of (4),

$$y(s; a, b) \geq y(s; a, b') \text{ for } s \leq a < b < b'. \quad (4')$$

(4) and (4') imply the existence of the limit

$$y(s; a) = \lim_{b \rightarrow +\infty} y(s; a, b) \quad (5)$$

at any $s \leq a$.

If in (3) α, β are both chosen less than a it becomes obvious that (5) exists at any s and that $y(s; a)$ is a solution of (1). It is also inferred that

$$y'(s; a) = \lim_{b \rightarrow +\infty} y'(s; a, b) \quad (5')$$

holds at any s . From (2) and (4) we get

$$y(a; a) = 1, \quad y(s; a) \geq 0$$

for any s . As $y(s; a)$ is a solution of (1) we even have $y > 0$ everywhere. The function

$$u(s) = \frac{y'(s; a)}{y(s; a)}$$

which, according to (3'), does not depend on a is thus continuous at every s . It is a solution of the Riccati equation

$$u'(s) + u^2(s) + K(s) = 0. \quad (6)$$

In this manner, a perfectly well-defined function u is obtained on any oriented geodesic of S . The value of this function is perfectly well de-

terminated at every point of the geodesic and is obviously independent of the choice of the point where s is counted from. u is an everywhere continuous solution of (6) along the geodesic.

Let us note for later use that $u(s)$ is the limit of

$$\frac{y'(s; a, b)}{y(s; a, b)}$$

as $b \rightarrow \infty$. This fraction equals

$$\frac{Y'(s; b)}{Y(s; b)}$$

where $Y(s; b)$ is the solution of (1) satisfying $Y = 0$, $Y' = 1$ at $s = b$, and thus we may write

$$u(s) = \lim_{n \rightarrow \infty} \frac{Y'_s(s; s+n)}{Y(s; s+n)}. \quad (7)$$

We next prove the boundedness of u for all geodesics on S . From the closedness of S one infers that

$$K > -A^2 \quad (8)$$

on S with a suitable constant $A > 0$. A Sturmian argument will show that

$$|u| \leq A. \quad (9)$$

Let

$$z(s; a, b) = \frac{\sinh A(b-s)}{\sinh A(b-a)}$$

be the solution of

$$z''(s) - A^2 z(s) = 0 \quad (10)$$

satisfying

$$z(a; a, b) = 1, \quad z(b; a, b) = 0.$$

From (1) and (10) we obtain

$$(zy' - yz')' = -(K + A^2)zy. \quad (11)$$

For $y = y(s; a, b)$ and $z = z(s; a, b)$, $a < b$, z and y are both positive for $s < b$. As $zy' - yz' = 0$ for $s = b$ we infer that $zy' - yz' > 0$ for $s < b$, i.e., that

$$u(s; b) = \frac{y'(s; a, b)}{y(s; a, b)} > \frac{z'(s; a, b)}{z(s; a, b)}$$

and therefore, on passing to the limit $b \rightarrow +\infty$, that

$$u(s) \geq -A.$$

On the other hand, $y = y(s; a)$ and $z = z(s; a, c)$, $c < a$, are both positive for $s > c$, and it follows from (11) that

$$(zy' - yz')_c < (zy' - yz')_c = -y(c)z'(c) < 0 \text{ for } s > c.$$

We therefore have, for $s > c$,

$$u(s) = \frac{y'(s; a)}{y(s; a)} < \frac{z'(s; a, c)}{z(s; a, c)}$$

and finally, on letting $c \rightarrow -\infty$, $u \leq A$.

We now consider the three-dimensional space Ω of the oriented line elements P situated on S . Let p denote the bearer point of P on S . Let, furthermore, P_t denote the line element obtained by moving P by arc length t tangentially along the geodesic through P . P_t depends continuously on P and t . For arbitrary fixed t , the transition from P to P_t is a one-to-one mapping of Ω into itself that is well known to leave the volume differential

$$dm = do d\varphi$$

invariant where do and $d\varphi$ denote the differentials of area and angle on S , respectively. Now, the function u constructed above is a bounded function of P , $u = u(P)$. For P fixed, $u(t) = u(P_t)$ is differentiable and satisfies the Riccati equation

$$\frac{du(P_t)}{dt} + u^2(P_t) + K(P_t) = 0 \quad (K(P) = K(p)). \quad (12)$$

$u(P)$ is easily shown to be measurable (by a somewhat longer argument one can even prove its continuity). Equation (7) can be written

$$u(P) = \lim_{n \rightarrow \infty} \frac{\frac{dY_n(s; P)}{ds}}{Y_n(s; P)} \bigg|_{s=0}, \quad (13)$$

where $Y_n(s; P)$ is the solution of the Jacobi equation

$$Y''(s) + K(P_s)Y(s) = 0$$

that vanishes at P_n , i.e., at $s = n$, with the derivative one. It follows from the well-known continuity theorem for the solution of the initial value problem (which applies here as S is of class C''') that $Y_n(s; P)$ and its derivative with respect to s are continuous functions of P for s fixed. As $Y_n < 0$ for $s < n$ the fraction on the right in (13) depends continuously

on P . As a limit function of a sequence of continuous functions $u(P)$ must be measurable. Together with the boundedness this implies the summability of $u(P)$ over Ω in the sense of the measure m .

From (12) we obtain on integrating with respect to t

$$u(P_1) - u(P) + \int_0^1 K(P_t) dt = \int_0^1 u^2(P_t) dt \quad (14)$$

for every P . Now, the invariance of the measure m under the mapping $P \rightarrow P_t$ implies the equality

$$\int_{\Omega} f(P_t) dm_P = \int_{\Omega} f(P) dm_P$$

for any summable function $f(P)$. By integration of (14) with respect to P and by using the last identity one obtains on the left-hand side

$$\begin{aligned} \int_{\Omega} \left[\int_0^1 K(P_t) dt \right] dm_P &= \int_0^1 \left[\int_{\Omega} K(P_t) dm_P \right] dt = \int_{\Omega} K(P) dm \\ &= 2\pi \int_S K(p) do. \end{aligned}$$

The order of integration may be changed since $K(P_t)$ depends continuously on P and t . The right-hand side of (14) must be a summable function of P . The resulting equality

$$2\pi \int_S K(p) do = - \int_{\Omega} \left[\int_0^1 u^2(P_t) dt \right] dm_P$$

immediately proves the validity of the theorem. If the total curvature is zero the right-hand side of (14) must be zero for almost all P . For every such P , $u(P_t)$ must vanish at every t , $0 \leq t \leq 1$, because it depends continuously on t . From (12) one infers that $K(P) = 0$ for such a P . As a continuous function K must therefore vanish everywhere on S .

* The German original of this note had been dedicated and presented to C. Carathéodory on his seventieth birthday on September 13, 1943. Though accepted for subsequent publication it never appeared in print. Its loss by an air raid did not become known to the author until long after the end of the war.

¹ Morse, M., and Hedlund, G. A., "Manifolds Without Conjugate Points," *Trans. Am. Math. Soc.*, **51**, 362-386 (1942).

² Hopf, E., "Statistik der Lösungen geodäetischer Probleme vom instabilen Typus," *Math. Annalen*, **117**, 590-608, in particular p. 608 (1940/41).

THE COMPLETENESS OF THE IRREDUCIBLE UNITARY REPRESENTATIONS OF A LOCALLY COMPACT GROUP

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The purpose of this note is to generalize some of the known results on unitary representations of compact and locally compact commutative groups to arbitrary separable locally compact groups. It is clear from such examples as the infinite cyclic group or the additive group of real numbers that unitary representations of non-compact groups in general do not decompose in a discrete manner. One needs a notion of generalized direct sum ("direct integral") of Hilbert spaces such as¹ von Neumann's² axiomatic characterization of generalized direct sums which he proves to be equivalent to the following:

Let $s(t)$ be a non-decreasing, right-semicontinuous, bounded, real-valued function of a real variable t and suppose that for each t a Hilbert space \mathfrak{H}_t of finite or countably infinite dimension $k(t)$ is given such that $k(t)$ is s -measurable. The Hilbert space \mathfrak{H} consisting of all s -measurable complex valued functions $x_n(t)$ ($n = 1, 2, \dots, k(t)$) satisfying

$$\sum_n \int |x_n(t)|^2 ds(t) < \infty$$

is von Neumann's generalized direct sum or direct integral of the spaces \mathfrak{H}_t with respect to the weight function $s(t)$: $\mathfrak{H} = \int \mathfrak{H}_t$. Thus to every $x \in \mathfrak{H}$ corresponds a function $x(t)$ with values in the various \mathfrak{H}_t : $x \sim x(t)$. We shall call $x(t)$ the *component* of x in \mathfrak{H}_t . If x is given then $x(t)$ is determined for³ almost all t and conversely. A bounded operator A in \mathfrak{H} can now be said to decompose if $Ax \sim A(t)x(t)$ where $A(t)$ is for each t a bounded operator in \mathfrak{H}_t . (Compare reference 2.) Let F be any family of bounded operators A in \mathfrak{H} and suppose each $A \in F$ decomposes into $A(t)$ under the given decomposition of \mathfrak{H} . Then we shall denote the family of operators $A(t)$ by $F(t)$.

THEOREM 1. *Let F be any self-adjoint family of bounded operators in a separable Hilbert space \mathfrak{H} . Then there exists a generalized direct sum $\mathfrak{H} = \int \mathfrak{H}_t$ under which every $A \in F$ decomposes such that almost³ all \mathfrak{H}_t are irreducible under $F(t)$, i.e., have no proper closed invariant linear subspaces.*

The proof consists in showing that the various decompositions of \mathfrak{H} into irreducible spaces \mathfrak{H}_t correspond exactly to the maximal commutative subrings of the ring F' of those operators which commute with every $A \in F$.

THEOREM 2. *Let U be a unitary representation of a group G in a separable*

Hilbert space \mathfrak{H} . Then U is generalized direct sum of irreducible unitary representations U_i . Suppose further that G is locally compact; then if U is continuous in the strong topology for operators⁶ so are almost³ all representations U_i .

If $g \in G$ and $U(g)$ represent g then under the decomposition $\mathfrak{H} = \int \mathfrak{H}_t$, the operator $U(g)$ is decomposed into operators $U(g, t)$ acting in \mathfrak{H}_t . According to reference 2 a change of $U(g, t)$ on a t -set of s -measure zero does not affect $U(g)$, thus to $U(g)$ corresponds for every g a class of (operator-valued) functions $U(g, t)$ and the proof consists in showing first that a representative can be chosen from each class in such a manner that the mapping $U(g) \rightarrow U(g, t)$ preserves the algebraic operations on operators, for all t outside a certain s -null-set which does not depend on g . Application of Fubini's theorem shows next that $U(g, t)$ is measurable (and hence continuous) in g for³ almost all t .

The above implies that the completeness relation which was established by H. Weyl⁴ for the space of square integrable functions on any coset-space of a compact group generalizes to any (separable) unitary representation space \mathfrak{H} of an arbitrary locally compact group as follows:

If $\mathfrak{H} = \int \mathfrak{H}_t$ is a decomposition of \mathfrak{H} into irreducible representation spaces \mathfrak{H}_t and if $y(t), z(t)$ are the components of y, z in the space \mathfrak{H}_t then

$$(y, z) = \int_{-\infty}^{\infty} (y(t), z(t)) ds(t), \quad (1)$$

where (y, z) denotes the inner product in \mathfrak{H} and $(y(t), z(t))$ the inner product in \mathfrak{H}_t .

In particular if \mathfrak{H} is the space $L_2(G)$ of square integrable functions $y(g), z(g), \dots$ on a separable locally compact group G and if $y(t), z(t)$ are the components of $y(g), z(g)$ in the (not necessarily finite dimensional) irreducible spaces \mathfrak{H}_t then

$$\int_G y(g) \overline{z(g)} dg = \int_{-\infty}^{\infty} (y(t), z(t)) ds(t).$$

Here dg refers to left- or right-invariant Haar measure on G according as left- or right-translations in $L_2(G)$ are considered. In the sense of this Parseval relation those irreducible unitary representations of G which occur in a decomposition of the regular representation are complete in the space $L_2(G)$.

Another consequence of Theorem 2 is that every Haar-measurable positive definite function $f(g)$ on a separable locally compact group G can be expressed for all g outside a certain set of Haar-measure zero in the form

$$f(g) = \int_{-\infty}^{\infty} f_t(g) ds(t),$$

where the $f_t(g)$ are "elementary" positive definite functions on G in the sense of reference 5 and $s(t)$ a function of the type considered above,

depending on $f(g)$. This follows at once if one uses the correspondence established by Gelfand and Raikov¹ between the unitary representations of G and the positive definite functions on G , together with relation (1) in the form

$$(U(g)y, y) = \int_{-\infty}^{\infty} (U(g, t)y(t), y(t))ds(t).$$

A detailed account of the above results will be published elsewhere.

¹ I am very much indebted to Professor John von Neumann for the privilege of having been able to read his manuscript on "Generalized Direct Sums" before its publication.

² von Neumann, John, "On Rings of Operators. Reduction Theory." To appear soon in *Ann. Math.*

³ I.e., for all t outside a certain set whose measure with respect to the weight function $s(t)$ is zero.

⁴ Weyl, Hermann, "Harmonics on Homogeneous Manifolds," *Ibid.*, 35, 486-499 (1934).

⁵ Gelfand, I., and Raikov, D., "Irreducible Unitary Representations of Locally Bi-compact Groups," *Compt. rend. acad. sci. U.R.S.S.*, 42, 199-201 (1944); *Rec. Math. (Math. Sbornik)*, 13 (55), 301-316 (1943).

⁶ von Neumann, John, "Zur Algebra der Funktionaloperatoren und Theorie der normalen Operatoren," *Math. Annalen*, 102, 370-427 (1929).

ON LACUNARY TRIGONOMETRIC SERIES, II

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This note is a continuation of another one with which the reader is supposed to be acquainted.¹ Except when otherwise stated, notation here is the same as before.

(vii) *Propositions (i)-(vi) remain valid even if the integral character of the numbers n_k in the lacunary series*

$$\sum_{k=1}^{\infty} (a_k \cos n_k x + b_k \sin n_k x), \quad n_{k+1}/n_k > q > 1 \quad (1)$$

is not assumed.

We shall confine our attention to (i) and (iii) which are typical. In the proof of (i) the integral character of the n_k played no rôle, so that the result obviously holds in the general case, even if instead of the distribution function of S_N/C_N in $(0, 2\pi)$ we consider the distribution function in any finite and fixed interval. As to (iii), its extension to non-integral n_k remains valid for every bounded set E of positive measure. The main difficulty in the proof of the extension is that the functions $\cos \gamma x$ which occur in the proof

of (iii) need not be orthogonal over any interval, which prevents us from using Bessel's inequality. To avoid this difficulty we may arrange the argument as follows.

First of all, a moment's consideration shows that it is enough to prove the result in the case when E is an interval. For then the result follows for E consisting of a finite or denumerable set of non-overlapping intervals, that is to say for open sets. Since every measurable set E is contained in an open set of measure arbitrarily close to $|E|$, we get the result for measurable sets. Thus let $E = (a, b)$, and let $K_\eta(x)$ be the familiar trapezoidal function equal to 1 in $(a + \eta, b - \eta)$, vanishing outside (a, b) and linear elsewhere. The area of the trapezoid we denote by k_η . If $Z_N(y)$ is the set of the points x at which $S_N(x)/C_N \leq y$, we define the function $H_N(y) = H_{N, \eta}(y)$ as

$$k_\eta^{-1} \int_{Z_N(y)} K_\eta(x) dx.$$

Clearly, $H_N(y)$ is a distribution function. It is enough to prove that it tends to $G(y) = (2\pi)^{-1/2} \int_{-\infty}^y \exp(-t^2/2) dt$, for then, making $\eta \rightarrow 0$, we obtain that the distribution function of $S_N(x)/C_N$ for (a, b) also tends to $G(y)$. For simplicity, we assume that (1) is a cosine series.

The distribution function of $H_N(y)$ is (see the proof of (iii))

$$\begin{aligned} \int_{-\infty}^{+\infty} e^{i\lambda y} dH_N(y) &= k_\eta^{-1} \int_a^b K_\eta(x) e^{i\lambda S_N(x)/A_N} dx = \\ &= e^{-1/2\lambda^2 k_\eta^{-1}} \int_a^b e^{i\lambda K_\eta(x)} \left\{ \prod_{k=1}^N \left(1 + \frac{i\lambda a_k}{A_N} \cos n_k x \right) \right\} e^{1/2\lambda^2 \xi_N} dx, \end{aligned}$$

where

$$\xi_N(x) = (2A_N^2)^{-1} \sum_{k=1}^N a_k^2 \cos 2n_k x.$$

The set of the points x in (a, b) at which $|\xi_N(x)| \geq \delta > 0$, is of measure

$$\leq \delta^{-2} \int_a^b \xi_N^2 dx \leq 1/4 \int_a^b A_N^{-4} \left[\sum_{k=1}^N (b-a) a_k^4 + \sum_{1 \leq k \neq l \leq N} 2a_k^2 a_l^2 |n_k - n_l|^{-1} \right]. \quad (2)$$

Since $|n_k - n_l|$ stays above a positive number for $k \neq l$, and is large if either k or l is large, the second sum in square brackets is obviously $o(A_N^4)$. Since the first sum is also $o(A_N^4)$, the whole expression (2) is $o(1)$. Thus, as $N \rightarrow +\infty$ and $\lambda = O(1)$, the characteristic function of $H_N(y)$ is, with an error $o(1)$,

$$e^{-1/2\lambda^2 k_\eta^{-1}} \int_a^b K_\eta(x) \prod_{k=1}^N \left(1 + \frac{i\lambda a_k}{A_N} \cos n_k x \right) dx = e^{-1/2\lambda^2 k_\eta^{-1}} I_N,$$

and it is enough to prove that $I_N \rightarrow k_\eta$. To show this we need the following

two lemmas whose proofs are almost identical with the proofs of Lemmas 1 and 2

LEMMA 1'. Let a be any non-negative number. The number of solutions of the double inequality $a \leq n_{k_1} + n_{k_2} + \dots + n_{k_p} \leq a + 1$ ($k_1 > k_2 > \dots > k_p$) is less than C^p , where $C = C(q)$.

LEMMA 2'. The number of solutions of $a \leq n_{k_1} + \dots + n_{k_p} - n_{k_{p+1}} \leq a + 1$ ($k_1 > \dots > k_p > k_{p+1}$) is less than C^{p+1} , where $C = C(q)$.

Let us now consider the formula

$$\prod_{k=1}^N \left(1 + \frac{i\lambda a_k}{A_N} \cos n_k x \right) = \sum_{\nu} \alpha_{\nu} \cos \nu x, \quad (3)$$

and let us set $\epsilon_k = |i\lambda a_k/A_N|$. Hence α_{ν} and ϵ_k depend also on N and λ . The numbers ν that occur in (3) are the non-negative, and in general non-integral, numbers of the form $\pm n_{k_1} \pm n_{k_2} \pm \dots \pm n_{k_p}$, with $k_1 > k_2 > \dots > k_p$. Let β_m denote the sum of the numbers $|\alpha_{\nu}|$ for $m < \nu \leq m+1$, and let $m = 0, 1, \dots$. As in the proof of (iii), we are led to consider equations of the form

$$n_{h_1} + n_{h_2} + \dots + n_{h_s} = \nu + n_{l_1} + n_{l_2} + \dots + n_{l_t}, \quad (4)$$

and we get for β_m the estimate $2^{-(p-1)} \sum \epsilon_{h_1} \dots \epsilon_{h_s} \epsilon_{l_1} \dots \epsilon_{l_t}$, where $p = s + t$, and the summation is extended over all the solutions of (4) with $m < \nu \leq m+1$. Arguing as before, we find that the β_m tend uniformly to 0 as $N \rightarrow \infty$ and $\lambda = O(1)$. Also $\alpha_0 - 1 \rightarrow 0$. On the other hand,

$$h_{\nu} = \int_a^b K_{\eta}(t) \cos \nu t \, dt = O(\nu^{-2}),$$

so that $|h_{\nu}| \leq M/(1 + \nu^2)$ for $\nu \geq 0$. Hence

$$I_N = \int_a^b K_{\eta}(t) \prod_{k=1}^N \left(1 + \frac{i\lambda a_k}{A_N} \cos n_k t \right) dt = k_{\eta} + (\alpha_0 - 1)k_{\eta} + \sum_{\nu > 0} \alpha_{\nu} h_{\nu},$$

$$|I_N - k_{\eta}| \leq |\alpha_0 - 1| k_{\eta} + M \sum_{m=0}^{\infty} \frac{\beta_m}{1 + m^2},$$

and this shows that $I_N \rightarrow k_{\eta}$. This completes the proof of the theorem.

Let us revert to the case when the n_k in (1) are positive integers satisfying an inequality $n_{k+1}/n_k > q > 1$. Instead of the partial sums S_N of the series (1) we may consider linear means of the partial sums. If we want to display the terms of the series we can always write the means in the form

$$\sigma_m(x) = \sum_{k=1}^{\infty} \alpha_{mk} (a_k \cos n_k x + b_k \sin n_k x) \quad (5)$$

where the index m tends to $+\infty$ (in a continuous or discontinuous way). We assume that all the series (5) converge almost everywhere, and this implies that

$$\sum_k \alpha_{mk}^2 (a_k^2 + b_k^2) < +\infty \text{ for every } m. \quad (6)$$

(This is not a restrictive condition since it is well known that if a lacunary trigonometric series is summable by any linear method of summability in a set of positive measure, the series must converge almost everywhere.) About the numbers α_{mk} we make only one assumption which will be retained in all what follows, namely that

$$(T) \quad \lim_{m \rightarrow \infty} \alpha_{mk} = 1 \text{ for } k = 1, 2, \dots$$

We also set

$$C_m^* = \left\{ \frac{1}{2} \sum_k \alpha_{mk}^2 c_k^2 \right\}^{1/2}. \quad (7)$$

(viii) Suppose that $C_m = \{ \frac{1}{2} (c_1^2 + \dots + c_m^2) \}^{1/2} \rightarrow \infty$, and that

$$\text{Max.}_{1 \leq j < \infty} \{ |c_j \alpha_{mj}| / C_m^* \} \rightarrow 0 \text{ as } m \rightarrow \infty. \quad (8)$$

Then the distribution function of $\sigma_m(x)/C_m^*$ over any set E , $|E| > 0$, tends to the Gaussian distribution with mean value zero and deviation 1.

The proof closely follows that of (iii). For, first of all, under condition (T) the divergence of $\sum c_m^2$ implies that $C_m^* \rightarrow \infty$ (and conversely). Arguing as in the proof of (iii) and confining ourselves for the sake of simplicity to purely cosine series (so that we can write a_m, A_m, A_m^* for c_m, C_m, C_m^*) we see that the distribution function of $\sigma_m(x)/A_m^*$ is

$$|E|^{-1} \int_E e^{i\lambda \sigma_m(x)/A_m^*} dx = o(1) \\ + e^{-\lambda^{1/2}} |E|^{-1} \int_E \prod_{k=1}^{\infty} \left(1 + i \frac{\lambda a_k \alpha_{mk}}{A_m^*} \cos n_k x \right) dx = o(1) + e^{-\lambda^{1/2}},$$

and the proof concludes as before.

Condition (8), though natural, is not quite simple, and we shall consider two special cases.

(a) Suppose that for each m the numbers α_{mk} are non-negative and decrease as k increases. Then condition (8) is satisfied if $c_j/C_j \rightarrow 0$.

For

$$c_j^2 \alpha_{mj}^2 / C_m^{*2} \leq 2c_j^2 \alpha_{mj}^2 / \sum_{k=1}^j \alpha_{mk}^2 c_k^2 \leq 2c_j^2 / C_j^2.$$

Thus the left-hand side here is small for j large, uniformly in m . If j is not large, the left-hand side is small as $m \rightarrow \infty$ since the numerator $c_j^2 \alpha_{mj}^2$ is $\leq c_j^2 \alpha_{mo}^2$ and the denominator C_m^{*2} tends to ∞ .

The assumptions of (a) are satisfied by the methods of Cesaro of positive order and by the method of Abel. Considering, for example, the latter we conclude that

(ix) If $c_m \rightarrow \infty$, $c_m/C_m \rightarrow 0$, and if we set $C(r) = \left\{ \frac{1}{2} \sum (a_k^2 + b_k^2) r^{2n_k} \right\}^{1/2}$,

$$f_r(x) = \sum_{k=1}^{\infty} (a_k \cos n_k x + b_k \sin n_k x) r^{n_k}$$

then, as $r \rightarrow 1$, the distribution function of $f_r(x)/C(r)$ over any set E , $|E| > 0$, approaches the Gaussian distribution with mean value 0 and deviation 1.

For $n_{k+1}/n_k \rightarrow \infty$, and $E = (0, 2\pi)$, see a paper by Kac.²

(b) Suppose that the numbers α_{mk} are uniformly bounded in m and k . Then condition (8) is satisfied for $c_j = O(1)$, $C_j \rightarrow \infty$.

For then the numerators in (8) are bounded and the denominators tend to $+\infty$, on account of condition (T). An example is provided by the Lebesgue method of summability which defines the sum of (1) as the limit of

$$\sum_{k=1}^{\infty} (a_k \cos n_k x + b_k \sin n_k x) \left(\frac{\sin n_k h}{n_k h} \right)$$

for $h = (1/m) \rightarrow +0$. Thus, if $c_k = O(1)$, $C_k \rightarrow \infty$, and if $F(x)$ is the sum of the series (1) integrated term by term (in view of the assumptions the series defining F converges absolutely and uniformly) then the distribution function over E of the differential ratio $\{F(x+h) - F(x-h)\}/2h$, divided by $\left\{ \frac{1}{2} \sum c_k^2 \left(\frac{\sin n_k h}{n_k h} \right)^2 \right\}^{1/2}$ approaches the standard Gaussian distribution.

The same result holds for the ratio $\{F(x+2h) - F(x)\}/2h$ (and is a corollary of the preceding result if E is an interval). A simple illustration is provided by the Hardy-Weierstrass non-differentiable function $F(x) = \sum a^{-n} \cos a^n x$, $a > 1$.

Obviously a result parallel to (viii) holds for the series (1) of the class L^2 . Since such series converge almost everywhere, we consider the distribution functions of the differences

$$\Delta_m(x) = f(x) - \sigma_m(x) = \sum_{k=1}^{\infty} \beta_{mk} (a_k \cos n_k x + b_k \sin n_k x)$$

where for each k the numbers $\beta_{mk} = 1 - \alpha_{mk}$ approach 0 as $m \rightarrow \infty$, on account of condition (T). Let us set

$$D_m = \left(\frac{1}{2} \sum_{k=m}^{\infty} c_k^2 \right)^{1/2}, \quad D_m^* = \left(\frac{1}{2} \sum_{k=1}^{\infty} \beta_{mk}^2 c_k^2 \right)^{1/2}.$$

If we want each of the expressions $\sigma_m(x)$, $\Delta_m(x)$ to have meaning (almost everywhere in x) we must assume that the numbers D_m^* are finite, which is a consequence of the convergence of $\sum c_k^2$ only if the elements of each row of the matrix $\{\alpha_{mk}\}$ are bounded. An obvious analogue of (viii) is

(x) If the series (1) is of the class L^2 , the D_m^* are finite, and

$$\text{Max.}_{1 \leq j < \infty} \{ |c_j \beta_{mj}| / D_m^* \} \rightarrow 0 \text{ as } m \rightarrow \infty, \quad (9)$$

then the distribution function of $\Delta_m(x)/D_m^*$ over any set E , $|E| > 0$, tends to the standard Gaussian distribution.

As in the case (a) above, condition (9) is a consequence of $c_m/D_m \rightarrow 0$ if the numbers α_{mk} are non-negative and decrease as k increases. In particular if $c_m/D_m \rightarrow 0$, the distribution function of $\{f(x) - f_r(x)\} / \{1/2 \Sigma c_k^2 (1 - r^{n_k})^2\}^{1/2}$ tends to the standard Gaussian distribution.

We shall now consider besides (1) another lacunary series

$$\Sigma (a_k' \cos m_k x + b_k' \sin m_k x), \quad m_{k+1}/m_k > q > 1, \quad (10)$$

whose partial sums we shall denote by $S_M'(x)$.

(xi) Suppose that $\Sigma c_k^2 = \infty$, $\Sigma c_k'^2 = \infty$, that $c_k = o(C_k)$, $c_k' = o(C_k')$ and that the combined sequence of the n_k and m_k is still lacunary. Then on every set $EC(0, 2\pi)$, $|E| > 0$, the expressions $S_N(x)/C_N$ and $S_M'(x)/C_M'$ are asymptotically independent as $N, M \rightarrow \infty$.

To define the notion of asymptotic independence of S_N/C_N and S_M'/C_M' over E , let $Z_N(\alpha, \beta)$ denote the set of points $x \in E$ such that $\alpha \leq S_N/C_N \leq \beta$, and let $Z_M'(\alpha', \beta')$ have a similar meaning. Let $Z_{NM}(\alpha, \beta; \alpha', \beta')$ be the set of points of E where the inequalities for S_N/C_N and S_M'/C_M' are satisfied simultaneously. The assertion of (xi) is that

$$\lim_{N, M \rightarrow \infty} \{ |Z_{NM}(\alpha, \beta; \alpha', \beta')| / |E| \} = \lim_{N \rightarrow \infty} \{ |Z_N(\alpha, \beta)| / |E| \} \lim_{M \rightarrow \infty} \{ |Z_M'(\alpha', \beta')| / |E| \}$$

for all $\alpha, \beta, \alpha', \beta'$, the existence of the limits on the right being assured by (iii).

It is enough to sketch the proof of (xi) since it is analogous to that of (vi). It is enough to assume that $\alpha = \alpha' = -\infty$. Let us consider the joint distribution function $F_{NM}(\xi, \eta)$ of S_N/C_N and S_M'/C_M' over E , that is $Z_{NM}(-\infty, \xi; -\infty, \eta)/|E|$. The characteristic function of F_{NM} is

$$\int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} e^{i(\lambda\xi + \mu\eta)} dF_{NM}(\xi, \eta) = |E|^{-1} \int_E e^{i(\lambda S_N(x)/C_N + \mu S_M'(x)/C_M')} dx.$$

Since we assume that the combined sequence of the n_k and m_k is lacunary the argument used in the proof of (vi) is applicable without change, and the last integral tends to $\exp. \{-1/2(\lambda^2 + \mu^2)\}$. Thus

$$F_{NM}(\xi, \eta) \rightarrow (2\pi)^{-1} \int_{-\infty}^{\xi} \int_{-\infty}^{\eta} e^{-1/2(\lambda^2 + \mu^2)} d\lambda d\mu = (2\pi)^{-1/2} \int_{-\infty}^{\xi} e^{-1/2\lambda^2} d\lambda \cdot (2\pi)^{-1/2} \int_{-\infty}^{\eta} e^{-1/2\mu^2} d\mu,$$

and the factors on the right are asymptotically equal to $|Z_N(-\infty, \xi)|/|E|$ and $|Z_M'(-\infty, \eta)|/|E|$.

Various extensions of (xi) are possible. We might compare the remainders of the series (1) and (10) if the series are of the class L^2 ; or the partial

sums of one series with the remainders of the other; we might consider linear means of the series; or, finally, we might extend the results to the case of several series, provided the sequence of all exponents is still lacunary. The proofs would not require new ideas.

We shall now consider the case when the n_k in (1) are any set of linearly independent numbers, and again we confine our attention to an extension of (iii). Thus the condition of lacunarity is dropped and the n_k need not increase with k . By the distribution function F_N of $S_N(x)/C_N$ we mean $\lim_{T \rightarrow \infty} (2T)^{-1} |Z_N(y; -T, +T)|$, where $Z_N(y; -T, T)$ denotes the set of points x of the interval $(-T, T)$ at which $S_N/C_N \leq y$. The function $F_N(y)$ exists except for an at most denumerable set of y 's, since this is true for the general almost periodic function in the sense of Bohr.³

(xii) If the n_k are linearly independent, then the distribution function $F_N(y)$ of S_N/C_N tends to the standard Gaussian distribution provided $C_N \rightarrow \infty$, $c_N = o(C_N)$.⁴

For let us again assume that we deal with a cosine series. Then

$$\int_{-\infty}^{+\infty} e^{i\lambda y} dF_N(y) = \lim_{T \rightarrow \infty} \frac{1}{2T} \int_{-T}^T e^{i\lambda S_N(x)/A_N} dx =$$

$$e^{-\lambda^2/2} \lim_{T \rightarrow \infty} \frac{1}{2T} \int_{-T}^T \prod_{k=1}^N \left(1 + \frac{i\lambda a_k}{A_N} \cos n_k x \right) dx + o_N(1) = e^{-\lambda^2/2} + o_N(1),$$

where $o_N(1)$ stands for a quantity small for N large, uniformly in T . The argument here is essentially the same as always. In particular, if $\xi_N(x)$ has the same meaning as before, and if $Z_N^*(\delta, -T, +T)$ denotes the set of x in $(-T, T)$ such that $|\xi_N(x)| \geq \delta$, then

$$\limsup_{T \rightarrow \infty} \frac{|Z_N^*(\delta, -T, T)|}{2T} = \limsup_{T \rightarrow \infty} \frac{1}{2\delta^2 T} \int_{-T}^T \xi_N^2 dx = \frac{1}{8\delta^2 A_N^4} \sum_{k=1}^N a_k^4,$$

and the last expression is small with $1/N$.

We conclude the note with a discussion of the behavior, for $N \rightarrow \infty$, of the sum

$$S_N(x) = a_1 f(n_1 x) + a_2 f(n_2 x) + \dots + a_N f(n_N x)$$

where $n_1 < n_2 < \dots$ are positive integers, the a_k are given, and $f(x)$ is a trigonometric polynomial with mean value zero. For the sake of brevity we assume that $f(x)$ is purely cosine,

$$f(x) = c_1 \cos lx + \dots + c_m \cos mx,$$

and write

$$(2\pi)^{-1} \int_0^{2\pi} f^2 dx = \frac{1}{2}(c_1^2 + \dots + c_m^2) = \frac{1}{2} C_N A_N = \left\{ \frac{1}{2} C(a_1^2 + \dots + a_N^2) \right\}^{1/2}$$

Thus $f^2(x) = \frac{1}{2}C + g(x)$, where $g(x)$ has mean value zero.

(xiii) If $A_N \rightarrow \infty$, $a_N/A_N \rightarrow 0$, and

$$n_{k+1}/n_k \geq g > m/l, \quad (11)$$

then the distribution of $S_N(x)/A_N$ over any set E , $|E| > 0$, tends to the standard Gaussian distribution.

We have to show that $|E|^{-1} \int_E \exp. (i\lambda S_N/A_N) dx \rightarrow \exp. (-\frac{1}{2}\lambda^2)$ as $N \rightarrow \infty$. Setting, as before,

$$\sum_1^N \frac{a_k^2}{A_N^2} f^2(n_k x) = 1 + \sum_1^N \frac{a_k^2}{A_N^2} g(n_k x) = 1 + \xi_N(x),$$

we must show that the set of points $x \in (0, 2\pi)$ where $|S_N/A_N| \geq \delta > 0$ is of measure tending to 0 as $N \rightarrow \infty$, and this will follow if we prove that $\int_0^{2\pi} \xi_N^2 dx \rightarrow 0$. To compute this integral one has to consider the integrals of the type $\int_0^{2\pi} g^2(n_k x) dx = \int_0^{2\pi} g^2(x) = G$, and of the type $I_{h,k} = \int_0^{2\pi} g(n_h x) g(n_k x) dx$ for $h < k$. Since $g(x)$ is a polynomial of order $2m$ and mean value zero, $I_{h,k} = 0$ if $n_k > 2mn_h$. Therefore, for each h the number of the non-vanishing $I_{h,k}$ does not exceed an integer $r = r(m, q)$. Since $|I_{h,k}| \leq G$, one gets

$$\int_0^{2\pi} \xi_N^2 dx \leq BG(a_1^4 + \dots + a_N^4)A_N^{-4} \rightarrow 0,$$

where $B = B(m, q)$. Arguing as in the proof of (iii), it remains to show that in the formula

$$\prod_1^N (1 + i\lambda a_k A_N^{-1} f(n_k x)) = \alpha_0^N + \sum \alpha_\nu^N \cos \nu x$$

we have $\alpha_0^N \rightarrow 1$, and $\alpha_\nu^N \rightarrow 0$ for $\nu > 0$.

LEMMA 1". The number of solutions of $m_1 n_{k_1} + m_2 n_{k_2} + \dots + m_p n_{k_p} = A$ when $l \leq m_i \leq m$, is $< D^p$, where $D = D(l, m, q)$.

LEMMA 2". The number of solutions of $m_h n_h - m_k n_k = A$, with m_h and m_k between l and m , is $< E = E(l, m, q)$.

The proof of Lemma 1" requires no explanation. In the proof of Lemma 2", writing $A > ln_h - mn_k > ln_h(1 - m/lq)$ we use the fact that $q > m/l$. The proof of (xiii) is now completed by observing that if $\epsilon_k = |i\lambda a_k/A_N|$ the contribution in α_ν^N of all solutions of $m_1 n_{h_1} + \dots + m_s n_{h_s} = \nu + m_1' n_{j_1} + \dots + m_t' n_{j_t}$ is at most

$$2^{-(p-1)} \sum \epsilon_{k_1} \dots \epsilon_{k_s} \epsilon_{j_1} \dots \epsilon_{j_t} |c_{m_1} \dots c_{m_s} c_{m_1'} \dots c_{m_t'}| \leq 2^{-(p-1)} (\text{Max. } |c_t|)^p \sum \epsilon_{h_1} \dots \epsilon_{h_s} \epsilon_{j_1} \dots \epsilon_{j_t}$$

where $p = s + t$. From this point the proof proceeds as before.

Remark. That condition (11) cannot be relaxed is seen on the example $f(x) = \cos x + \cos mx$, $n_k = m^k - 1$. For then

$$\cos mn_k x + \cos n_{k+1}(x) = \cos \left(m^k - \frac{m+1}{2} \right) x \cos \frac{1}{2} (m-1)x$$

and combining the second term of $f(n_k x)$ with the first term of $f(n_{k+1} x)$ we see that the Gaussian distribution is altered by the factor $\cos \frac{1}{2}(m-1)x$. This remark is essentially due to Erdős.

¹ These PROCEEDINGS, **33**, 333-338 (1947).

² *Amer. J. Math.*, 1939.

³ Wintner, A., *Am. J. Math.*, **54**, 339-345 (1932); see also Jessen, B., *J. London Math. Soc.*, **8**, 247-250 (1933).

⁴ Compare Kac and Steinhaus, *Studia Mathematica*, **4**, 11 (1938).

APPLICATIONS OF CYCLOTOMY TO THE THEORY OF NON-HOMOGENEOUS EQUATIONS IN A FINITE FIELD

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In another paper¹ we obtained the following result:

"If p is an odd prime belonging to the exponent l modulo l and such that $p^l = 1 + cl$ with c and l prime to each other and l odd. Write $\theta = \zeta\beta$, where ζ is a primitive l -th root of unity and β is a primitive c -th root of unity. Let g be a primitive root of a finite field $F(p^l)$ of order p^l , and denote by $[v, r]$ the number of distinct solutions g^a, g^r of

$$1 + g^{v+ca} = g^{r+\gamma l}, \quad (1)$$

a in the set $0, 1, \dots, l-1$ and γ in the set $0, 1, \dots, c-1$. Associated with this number (v, r) is the cyclotomic number

$$\psi_{a,b}(\theta) = \sum_{i,j} [i, j] \beta^{bi} \zeta^{aj}, \quad (2)$$

with $a \not\equiv 0 \pmod{l}$, $b \not\equiv 0 \pmod{c}$, i ranging over the set $0, 1, \dots, c-1$ and j ranging over the set $0, 1, \dots, l-1$. Said function has the property

$$\psi_{a,b}(\theta) \psi_{a,b}(\theta^{-1}) = p^l. \quad (3)''$$

In the statement of this result in the former paper we assumed that l was prime. This restriction was not necessary and was not employed in the proof of the theorem. We also stated in connection with this theorem that certain quadratic relations governing the solution of (1) could be developed with the use of (2) and (3). In the present paper we shall derive said quadratic relations and also consider their connection with some results in another paper.²

In view of (3) we have

$$\sum_{i', j'} [i', j'] \beta^{bi'} \zeta^{aj'} \sum_{i, j} [i, j] \beta^{-bi} \zeta^{-aj} = p',$$

where i, i' , range independently over $0, 1, \dots, c-1$ and j, j' independently over $0, 1, \dots, l-1$, whence

$$\sum_{i', j', i, j} [i', j'] [i, j] \beta^{b(i'-i)} \zeta^{a(j'-j)} = p'. \quad (4)$$

Set $i' - i = h$ and $j' - j = k$. Then (4) becomes

$$\sum_{i, j, h, k} [i+h, j+k] [i, j] \beta^{bh} \zeta^{ak},$$

h ranging independently over $0, 1, \dots, c-1$, and k independently over $0, 1, \dots, l-1$.

Set

$$A_k = \sum_{i, j, h} [i+h, j+k] [i, j] \beta^{bh}.$$

Then (4) gives

$$A_0 + \zeta^a A_1 + \zeta^{2a} A_2 + \dots + \zeta^{(l-1)a} A_{l-1} = p'. \quad (5)$$

We may take $a = 1, 2, \dots, l-1$ and obtain $l-1$ equations. Also we have

$$A_0 + A_1 + A_2 + \dots + A_{l-1} = 1, \quad (6)$$

since it is the product of

$$\sum_{i, j} [i, j] \beta^{bi} \text{ and } \sum_{i', j'} [i', j'] \beta^{-bi'}$$

and each of these is (-1) . Making an array of the l equations obtained from (5) and (6) and eliminating β therefrom by the use of a method of a previous paper,³ gives

$$lA_0 = (l-1)p' + 1,$$

or

$$A_0 = cl - c + 1 = p' - c \quad (6a)$$

and

$$A_s = -c, \quad s = 1, 2, \dots, l-1. \quad (6b)$$

Set, if i and j range as before,

$$B_{h,k} = \sum [i+h, j+k] [i, j]. \quad (7)$$

We shall now show that

$$B_{0,k} + B_{1,k} + B_{2,k} + \dots + B_{c-1,k} = c^2l - 2c + 1 \quad (8)$$

for $k = 0$. By the definition (7) the left-hand member of this equals

$$\sum_{i,j,h} [i+h, j][i, j] = \sum_{i,j} ([i, j][i, j] + [i+1, j][i, j] + \dots + [i+c-1, j][i, j]). \quad (9)$$

We have, by keeping v fixed in (1) and counting the number of possible values of γ ,

$$\sum_{r=0}^{l-1} [v, r] = l \text{ if } v \neq \frac{c}{2}, \quad (10)$$

and

$$\sum_{r=0}^{l-1} \left[\frac{c}{2}, r \right] = l - 1. \quad (11)$$

Similarly

$$\sum_{v=0}^{c-1} [v, r] = c \text{ if } r \neq 0, \quad (12)$$

and

$$\sum_{v=0}^{c-1} [v, 0] = c - 1. \quad (13)$$

The expressions $i, i+1, i+2, \dots, i+c-1$ are congruent in some order to $0, 1, 2, \dots, c-1$ modulo c ; and hence by (12) we have for a fixed $j \neq 0$ the sum of the corresponding terms of (9) equals $\sum_{i,j} c[i, j]$; while for $j = 0$ the sum of the corresponding terms of (9) is by (13) equal to $\sum_i (c-1)[i, 0]$; whence (9) equals, ($j \neq 0$),

$$\sum_{i,j} c[i, j] + \sum_i (c-1)[i, 0] = \quad (14)$$

$c^2(l-1) + (c-1)^2$ or $c^2l - 2c + 1$ by again applying (12) and (13). But this is the conclusion stated in (8).

For $k \neq 0$, we have

$$\begin{aligned} & \sum_{h,i,j} [i+h, j+k][i, j] \\ &= \sum_{i,j} [i, j]([i, j+k] + [i+1, j+k] + \dots + [i+c-1, j+k]) \\ &= \sum_{i,j \neq l-k} [i, j]c + \sum_i (c-1)[i, l-k] \\ &= c \sum_{j \neq l-k} ([0, j] + [1, j] + \dots + [c-1, j]) + \sum_i (c-1)[i, l-k] \\ &= c(c-1) + c^2(l-2) + c(c-1) = c^2l - 2c. \end{aligned}$$

Or for $k \neq 0$,

$$\sum_{h,i,j} [i+h, j+k][i, j] = c^2l - 2c. \quad (15)$$

Employing (6a) and (14) we may write

$$\begin{aligned} B_{00} + B_{10} + \dots + B_{c-1,0} &= c^2(l-1) + (c-1)^2, \\ B_{00} + \beta B_{10} + \dots + \beta^{c-1} B_{c-1,0} &= p^t - c, \\ &\dots\dots\dots \\ &\dots\dots\dots \\ B_{00} + \beta^{c-1} B_{10} + \dots + \beta^{(c-1)(c-1)} B_{c-1,0} &= p^t - c. \end{aligned}$$

Also for $k \neq 0$, we have using (6b) and (15),

$$\begin{aligned} B_{0k} + B_{1k} + \dots + B_{c-1,k} &= c^2l - 2c \\ B_{0k} + \beta B_{1k} + \dots + \beta^{c-1} B_{c-1,k} &= -c \\ &\dots\dots\dots \\ &\dots\dots\dots \\ B_{0k} + \beta^{c-1} B_{1k} + \dots + \beta^{(c-1)(c-1)} B_{c-1,k} &= -c. \end{aligned}$$

Whence, employing the same method we used to obtain (6a) and (6b) from (5) and (6),

$$\left. \begin{aligned} B_{00} &= 2lc - l - c, \\ B_{h0} &= cl - l - 1 \text{ for } h \not\equiv 0 \pmod{c}, \\ B_{0k} &= cl - c - 1 \text{ for } k \not\equiv 0 \pmod{l}, \\ B_{hk} &= cl - 1 \text{ for } h \not\equiv 0 \pmod{c}, k \not\equiv 0 \pmod{l}. \end{aligned} \right\} \quad (16)$$

Whence the

THEOREM I, if p is an odd prime belonging to the exponent t modulo l and such that $p^t = 1 + lc$ with c and l prime to each other and l odd, and also g as a primitive root in a finite field $F(p^t)$. Denote by $[v, r]$ the number of distinct solutions g^a, g^r of the equation

$$1 + g^{v+ca} = g^{r+l\gamma},$$

where α is in the set $0, 1, \dots, l-1$ and γ in the set $0, 1, \dots, c-1$. Also set

$$B_{h,k} = \sum_{i,j} [i+h, j+k][i, j].$$

Then the relations (16) follow.

In the relation (1) we took for the exponent c and l to have the property that $cl = p^t - 1$. However, we might have taken c_1 and l_1 in place of c and l in (1) where $(c_1, l_1) = 1$, and $c_1 l_1$ divides $p^t - 1$, then if $\{i, j\}$ denotes the number of distinct solutions of the equation

$$1 + g^{i+c_1\delta} = g^{j+l_1\epsilon},$$

then we may use in lieu of (2) the number

$$\psi_{a,e}(\theta_1) = \sum_{i,j} \{i, j\} \beta_1^{di} \zeta_1^{ej},$$

where β_1 is a primitive c_1 -th root of unity and ζ_1 is a primitive l_1 -th root of

unity. Also θ_1 is a primitive in (c_1h) -th root of unity. This function also has the same property given in (3) and we may obtain relations analogous to (16) involving the quantities $\{i+h, j+k\}\{i, j\}$. As we were principally interested in the case where $cl = p^l - 1$ in (1), in view of certain applications, we did not carry out this more general investigation in detail.

In a former paper² we examined the number (i, j) of solutions g^r, g^s of

$$1 + g^{i+rl} = g^{j+sl},$$

where r and s are each in the range of $0, 1, \dots, c-1$ and obtained quadratic relations involving the quantities $(i, j)(j+h, j+k)$, given in Theorem I of that paper. The relations seem to be of somewhat different character from those of the present paper and we do not yet have a unified theory to cover the case where c and l are any integers in (1) of such a nature as to yield as special cases relations (16) of this paper as well as (23), (24), (25), (26) of the former paper.

¹ These PROCEEDINGS, **32**, 317 (1946).

² *Ibid.*, **33**, 236-242 (1947).

³ *Ibid.*, **31**, 189 (1945).

A SPECIAL CLASS OF SOLUTIONS OF THE EQUATIONS OF THE GRAVITATIONAL FIELD ARISING FROM CERTAIN GAUGE-INVARIANT ACTION PRINCIPLES

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1. By direct consideration of the variation of the integrals

$$J_1 = \int G^2 \sqrt{-g} d\tau, \quad J_2 = \int G^{\mu\nu} G_{\mu\nu} \sqrt{-g} d\tau$$

the author has shown¹ that in a V_4 the field equations arising out of the action principles $\delta J_1 = 0, \delta J_2 = 0$ are satisfied by any solution of the equations

$$G_{\mu\nu} = \alpha g_{\mu\nu}, \tag{1}$$

where α is an arbitrary constant, and $G_{\mu\nu}$ is the Ricci tensor. Let $\bar{B}_{\mu\nu\alpha}{}^\beta$ be the gauge-invariant curvature tensor of Weyl's theory;² and let $\bar{B}_{\mu\nu\alpha}{}^\alpha = \bar{G}_{\mu\nu}$, $g^{\mu\nu} \bar{G}_{\mu\nu} = \bar{G}$, so that in a region free from electromagnetic radiation $\bar{G}_{\mu\nu} = G_{\mu\nu}$, $\bar{G} = G$. Then by a slight extension of the method referred to above we may prove the following:

THEOREM. *In a region free from radiation ($\kappa_\mu = 0$) the field equations arising from a gauge-invariant action principle in which the Lagrangian is composed of the components of the contracted curvature tensor and the components of the metrical tensor are satisfied by any solution of the equations $G_{\mu\nu} = \alpha g_{\mu\nu}$, where α is an arbitrary constant.*

2. Defining the "absence of radiation" to mean

$$\kappa_\mu = 0, \quad (\mu = 1, 2, \dots, n) \quad (2)$$

we may prove the theorem somewhat more generally for a V_n . When (2) applies every action principle of the kind under consideration reduces to the form

$$\delta \int L(K_1, K_2, \dots, K_m) \sqrt{-g} d\tau = 0 \quad (3)$$

where the Lagrangian is some function of the $K_s = G_{\mu_1}^{\mu_2} G_{\mu_2}^{\mu_3} G_{\mu_3}^{\mu_4} \dots G_{\mu_n}^{\mu_1}$, ($K_1 = G$), and m is a positive integer. Equation (3) is the degenerate form of the action principle $\delta \int \tilde{L} \sqrt{-g} d\tau = 0$, where the Lagrangian \tilde{L} is composed of the $\tilde{G}_{\mu\nu}$ and the $g^{\mu\nu}$. In a gauge transformation in which $g_{\mu\nu}$ becomes $\lambda^2 g_{\mu\nu}$, $\sqrt{-g}$ becomes $\lambda^{+n} \sqrt{-g}$ and $\tilde{G}_{\mu\nu}$ remains unchanged, so that \tilde{L} must change into $\lambda^{-n} \tilde{L}$ since $\tilde{L} \sqrt{-g}$ is to be a gauge-invariant density. Putting $\kappa_\mu = 0$ after the transformation has been carried out this implies the condition

$$L(\mu K_1, \mu^2 K_2, \dots, \mu^m K_m) = \mu^{n/2} L(K_1, K_2, \dots, K_m), \quad (4)$$

where we have set $\mu = \lambda^{-2}$.

Differentiating (4) with respect to μ and putting $\mu = 1$ after the differentiation, this gives

$$\sum_{s=1}^m s K_s \frac{\partial L}{\partial K_s} = \frac{1}{2} n L. \quad (5)$$

Now

$$\delta L = \sum_{s=1}^m \frac{\partial L}{\partial K_s} \delta K_s; \quad (6)$$

and in view of the symmetry of K_s with respect to the $G_{\mu_i}^{\mu_{i+1}}$ we have

$$\delta K_s = s G_{\mu_1}^{\mu_2} G_{\mu_2}^{\mu_3} \dots G_{\mu_{s-1}}^{\mu_s} \delta G_{\mu_s}^{\mu_1}.$$

If now $G_{\mu\nu}$ be given by (1) it follows immediately that

$$K_s = \text{const.} = n\alpha^s, \quad \delta K_s = s\alpha^{s-1} \delta G = (sK_s/n\alpha) \delta G.$$

Hence, from (6),

$$\delta L = \frac{1}{n\alpha} \sum_{s=1}^m s K_s \frac{\partial L}{\partial K_s} \delta G = L \delta G / 2\alpha,$$

by (5). Therefore

$$\begin{aligned}\delta \int L \sqrt{-g} d\tau &= \int \left(\frac{1}{2\alpha} L \sqrt{-g} \delta G + L \delta \sqrt{-g} \right) d\tau \\ &= (L/2\alpha) \int (\delta(\sqrt{-g}G) - G \delta \sqrt{-g} + 2\alpha \delta \sqrt{-g}) d\tau,\end{aligned}$$

or

$$\delta \int L \sqrt{-g} d\tau = (L/2\alpha) \delta \int (G - (n-2)\alpha) \sqrt{-g} d\tau. \quad (7)$$

Accordingly the condition that the right-hand side of (7) should vanish is always compatible with equation (1) whereby it was transformed into the condition expressed by the vanishing of the right-hand side of (7). For²

$$\delta \int (G - (n-2)\alpha) \sqrt{-g} d\tau = \int (1/2 g^{\mu\nu} G - G^{\mu\nu} - 1/2 (n-2)\alpha g^{\mu\nu}) \sqrt{-g} \delta g_{\mu\nu} d\tau = 0,$$

by (1). Hence $\delta \int L \sqrt{-g} d\tau$ vanishes, and the theorem of section 1 is proved.

In particular the result applies to the case where the action is taken to be the generalized volume $\int \sqrt{-\det|\bar{G}_{\mu\nu}|} d\tau$, so that

$$L = \sqrt{\det|\bar{G}_{\mu\nu}|/g} \equiv (\sum a_{r_1} \dots a_{r_n} K_1^{r_1} K_2^{r_2} \dots K_n^{r_n})^{1/2}, \quad (\sum_{r=1}^n r \nu_r = n),$$

where the a 's are certain numerical coefficients. This clearly is of a form satisfying the condition (5).

¹ Buchdahl, H. A., *Proc. Edinburgh Math. Soc.* (in the press) (1948).

² Eddington, A. S., *The Mathematical Theory of Relativity*, Cambridge University Press, 1930, p. 204.

³ Reference 2, p. 139.

PHYSICAL FAMILIES OF CURVES IN SPACE

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1. In preceding papers, we studied physical families of curves in the plane. We gave characteristic properties and discussed the transformation theory. In the present paper, we shall generalize this theory¹ to space of three dimensions.

The important physical families of curves connected with an arbitrary (positional) field of force¹ are (1) trajectories, that is, the paths of motion of a particle; (2) brachistochrones, that is, the curves in a general (con-

servative) field of force along which the time of the constrained motion between any two points is least; (3) general catenaries, that is, the curves formed from the resulting equilibrium when an inextensible flexible string is suspended in the field; and finally (4) velocity systems, that is, a curve is a velocity curve corresponding to the speed v_0 , provided a particle starting from any lineal element of the curve with that speed describes a trajectory osculating the curve.

2. In the plane, we have shown that these four systems of physical curves may all be obtained as special cases of the following general problem.² To find curves along which a constrained motion is possible such that the pressure P is proportional to the normal component N of the force. Thus a *system of curves* S_k for an arbitrary field of force is defined by the relation $P = kN$.

Each of these systems S_k is a three-parameter family and is defined by a differential equation of third order

$$(\psi - y'\phi)y''' = [(\psi_x + y'\psi_y) - y'(\phi_x + y'\phi_y)]y'' - \left[3\phi + \frac{(n-2)(\phi + y'\psi)}{1 + y'^2} \right] y'^{1/2}, \quad (1)$$

where $n = 2/(k + 1)$. Of course (ϕ, ψ) are the rectangular components of the force vector and primes denote differentiation with respect to x .

3. Now we begin the investigation of the corresponding theory in space of three dimensions. A *system of curves* S_k in an arbitrary field of force with rectangular components (ϕ, ψ, χ) consists of curves along which a constrained motion is possible such that the osculating plane at each point contains the force vector and the pressure P is proportional to the normal component N of the force so that $P = kN$.

The tangential component T and the normal component N (along the principal normal) of the force vector are defined by the formulae

$$T = \frac{\phi + y'\psi + z'\chi}{(1 + y'^2 + z'^2)^{1/2}}, \quad N = \left[\frac{(\psi - y'\phi)^2 + (\chi - z'\phi)^2 + (y'\chi - z'\psi)^2}{1 + y'^2 + z'^2} \right]^{1/2} \quad (2)$$

provided the force vector lies in the osculating plane. The radius of curvature r of a curve is

$$r = \frac{(1 + y'^2 + z'^2)^{1/2}}{[y''^2 + z''^2 + (y'z'' - y''z')^2]^{1/2}}. \quad (3)$$

If the particle is assumed to be of unit mass, the pressure P , along the principal normal to the path of the particle, is

$$P = \frac{v}{r^2} - N. \quad (4)$$

Along a curve of the system S_k , we must have $P = kN$. This condition $P = kN$ is equivalent to the two equations

$$P = \frac{v^2}{r} - N = kN, \quad v \frac{dv}{ds} = T, \quad (5)$$

where v denotes speed and s arc length. Eliminating the speed v from these two relations, we find the condition

$$N \frac{dr}{ds} = nT - r \frac{dN}{ds}, \quad (6)$$

where $n = \frac{2}{k+1}$.

Using the condition that the force vector lies in the osculating plane, we find from (2) and (3) that (6) reduces to the form

$$\frac{d}{dx} \left[\frac{1}{y''} (\psi - y'\phi)(1 + y'^2 + z'^2) \right] = n(\phi + y'\psi + z'\chi). \quad (7)$$

Simplifying this together with the condition that the force vector lies in the osculating plane, we find that the differential equations defining any system S_k in an arbitrary field of force, are

$$\begin{aligned} (\psi - y'\phi)y''' &= [(\psi_r + y'\psi_\nu + z'\psi_z) - y'(\phi_r + y'\phi_\nu + z'\phi_z)]y'' - \\ &\quad \left[3\phi + \frac{(n-2)(\phi + y'\psi + z'\chi)}{1 + y'^2 + z'^2} \right] y''^2, \quad (8) \\ z'' &= \frac{\chi - z'\phi}{\psi - y'\phi} y''. \end{aligned}$$

A system S_k in space consists of a five-parameter family of curves. Thus there are ∞^5 curves in a given S_k . The relation between k and n is

$$n = \frac{2}{k+1}. \quad (9)$$

It is found that the four cases of physical interest are

$k = 0$ or $n = 2$ gives S_0 , the system of trajectories.

$k = -2$ or $n = -2$ gives S_{-2} , the system of brachistochrones.

$k = 1$ or $n = 1$ gives S_1 , the system of general catenaries.

$k = \infty$ or $n = 0$ gives S_∞ , the system of velocity curves.

4. We find that any system S_k of ∞^5 curves possesses the following Geometric Properties I and II.³

I. The osculating planes of the ∞^3 curves passing through a given point form a pencil; that is, all the planes pass through a fixed direction.

II. The osculating spheres of the ∞^1 curves passing through a given point

in a given direction form a pencil; their centers thus describe a straight line.

5. We also find that the associated plane systems, determined by the given space system in the manner described below, have the five geometric properties characteristic of a planar system S_k' .

Consider the given system S_k of ∞^3 space curves in connection with any plane p . Through any point of p there pass ∞^2 curves of the given system which are tangent to the plane. Project the differential elements of the third order belonging to these space curves orthogonally upon p , thus obtaining ∞^2 plane differential elements of the third order at the selected point. Applying this process to all points of p , we have a defined set of ∞^4 differential elements of the third order. These elements define a certain differential equation of the third order, and thus determine a system of ∞^3 integral curves. This we term the associated system in the plane p . The space system S_k has the property that every one of these plane systems associated with it is a planar system S_k' .

6. In the space system S_k , consider the curves which pass through a given point in the direction of the line of force. We find that all of these have zero curvature except one particular curve. The ratio ρ of the curvature of this one particular curve of the system S_k to the curvature of the tangent line of force is

$$\rho = \frac{1}{n+1} = \frac{k+1}{k+3}. \quad (10)$$

For the four cases of physical interest, this ratio ρ is as follows: (1) For the system S_0 of trajectories, we have $\rho = 1/3$. This is the Theorem of Kasner on the ratio of departures of the rest trajectory and the line of force from their common tangent line. (2) For the system S_{-2} of brachistochrones, we find $\rho = -1$. (3) For the system S_1 of general catenaries, we obtain $\rho = 1/2$. (4) For the system S_∞ of velocity curves, we get $\rho = 1$.

7. Finally we discuss the transformation theory of spacial systems S_k . We have established the following result.⁴

Except for the system S_0 of trajectories, the only point transformations which convert every system of curves S_k into a system S_k are the similitudes which form a seven-parameter group in space. All the point transformations of space which send every system S_0 of trajectories into a system S_0 of trajectories form the fifteen-parameter collineation group.

In particular, we find that the fundamental two-dimensional projective theory given by Appell (1889) can be extended to three dimensions.

Later we shall discuss the corresponding theory for generalized fields of force where the field of force depends not only on the position of the point but also on the direction at the point.

¹ Kasner, "Differential-Geometric Aspects of Dynamics," Princeton Colloquium Lectures, *Am. Math. Soc. Publications*, 1913 (1934) (1948).

² Kasner, "Physical Curves," these PROCEEDINGS, 33, 246-251 (1947).

³ Kasner, "The Trajectories of Dynamics," *Trans. Am. Math. Soc.*, 7, 401-424 (1906). Also, "Dynamical Trajectories: The Motion of a Particle In An Arbitrary Field Of Force", *Trans. Am. Math. Soc.*, 8, 135-158 (1907).

⁴ For the plane transformation theory, see Kasner and De Cicco, "Transformation Theory of Physical Curves," these PROCEEDINGS, 33, 338-342 (1947).

A PHYSIOLOGICAL BASIS FOR SOME SUPPRESSOR MUTATIONS AND POSSIBLY FOR ONE GENE HETEROSIS*

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A mutant strain of *Neurospora* which requires sulfonamides for growth at 35° frequently becomes altered so that it can grow at that temperature without sulfonamides.¹ In each instance analyzed, the developed ability to grow without sulfonamides has resulted from mutation of genes distinct from that responsible for the sulfonamide requirement. Each "reverted" strain has proved to be a heterocaryon, composed of two kinds of nuclei, both of which carry the gene (*sfo*) for sulfonamide requirement, but one also carries a new "suppressor" gene. At least two independent suppressor genes have been involved in such reversions. Homocaryotic strains have been isolated, some carrying both *sfo* and the suppressor, others carrying the suppressor alone. None of these isolates has the growth characteristics of wild type, but each shows some peculiar relationship to the *p*-aminobenzoic acid requirement, the nature of which is still obscure. In a few instances, artificially constructed heterocaryons between the sulfonamide-requiring strain and a strain carrying both the sulfonamide-requiring gene and a suppressor have resulted in better growth on minimal medium than either strain is capable of by itself, thus reconstituting the situation observed in the original "reverted" heterocaryons.

As long as it was believed that sulfonamides were used as metabolites by the sulfonamide-requiring strain it was difficult to picture a possible physiological basis for the circumvention of the drug requirement by mutation of some entirely different gene. Recently Zalokar² has shown that the "sulfonamide-requiring" strain can grow in the absence of sulfonamides provided the available *p*-aminobenzoic acid is reduced to a particular concentration, growth being inhibited by both higher and lower

concentrations. It now appears that in the sulfonamide-requiring strain some reaction catalyzed by *p*-aminobenzoic acid is detrimental in that growth is prevented unless the effective amount of *p*-aminobenzoic acid is decreased, either by the competition of sulfonamides,¹ or by a reduction in the amount synthesized by the strain.² It follows then that any mutation

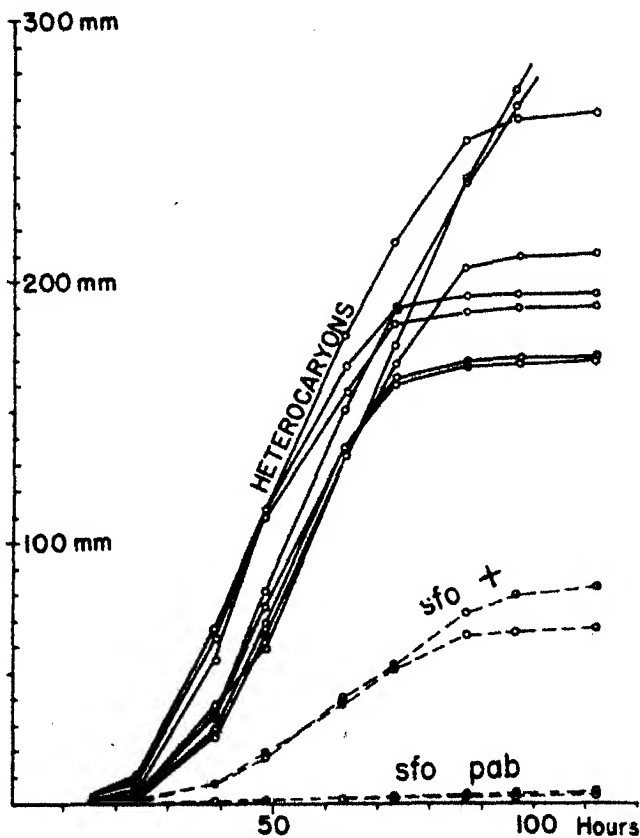


FIGURE 1

Growth curves of the sulfonamide-requiring mutant strain (*sfo*, +), the double mutant sulfonamide requiring, *p*-aminobenzoicless (*sfo*, *pab*), and of heterocaryons between them, on minimal medium at 35°. Changes in growth rates in the heterocaryons are presumably due to changing relative frequencies of the two types of nuclei at the growing tips.

which would have the effect of reducing the available *p*-aminobenzoic acid content might permit growth of the sulfonamide-requiring strain in the absence of sulfonamides, and this could happen even if the mutant gene was carried by only part of the nuclei in a heterocaryon.

To test this possibility, artificial heterocaryons were made between a double mutant strain carrying the sulfonamide-requiring gene (*sfo*) and a gene (*pab*) which prevents the synthesis of *p*-aminobenzoic acid, and a strain carrying *sfo* and the wild type allele (+) of *pab*. Neither of these strains *sfo*, *pab* or *sfo*, + grows appreciably on minimal medium at 35°, but as indicated in figure 1 the heterocaryons between them do.

These are not the ordinary sort of heterocaryons (cf. Beadle and Coonradt³) in which growth results because each of the two sorts of nuclei carries the wild type allele of the mutant gene carried by the other, since in these there are no nuclei carrying the wild type allele of *sfo*. In this case growth results from a balance between the production of *p*-aminobenzoic acid by one type of nucleus and the lack of production by the other to give an amount tolerated by strains carrying *sfo*, yet still sufficient for growth.

A heterocaryon of this sort, composed of two kinds of haploid nuclei, both carrying *sfo*, and one carrying *pab* the other +, is roughly equivalent to a diploid organism heterozygous for a single pair of alleles *pab*/+, the homocaryotic strains mixed to form the heterocaryons can be compared to the corresponding homozygous diploids *pab/pab* and +/+. The augmented growth of the heterocaryon reminds one of the instances of single gene heterosis in maize reported by Jones.⁴

It is not the intent of this note to suggest that most instances of one gene heterosis and most occurrences of suppressor mutations result from competitive systems such as must be involved in the case just described, but such a possibility must be borne in mind.

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A CYTOCHEMICAL STUDY OF THE FEULGEN NUCLEAR
REACTION

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The growing interest in the study of the chemical composition of cellular formed elements has led to wide-spread research on techniques for demonstrating and estimating, *in situ*, the presence and amounts of the various components found by macrochemical analysis. In short, this would involve the use of a quantitative chemical reaction and a means of accurately measuring the end-product of the reaction. Such techniques for measuring nucleoproteins of cell nuclei were recently described by Pollister and Ris.¹ Using these same methods, a study of the nature of the Feulgen nuclear reaction was undertaken.

The Feulgen reaction may be considered as occurring in two steps: first, the splitting of the linkage between the purine bases and the desoxypentose of the nucleic acid by means of mild acid hydrolysis, and second, a chemical reaction between the aldehyde groups, thus formed on the sugar, and the Schiff reagent (decolorized fuchsin) resulting in the synthesis of a new dye compound. It was noticed by Bauer,² and confirmed by Hillary,³ that the intensity of color produced in chromatin of cell nuclei is related to hydrolysis in such a way that as time of hydrolysis is increased there is a more and more intense staining until a maximum point is reached; while beyond this maximum, longer hydrolysis results in a decreased staining. The increased staining could easily be explained by the progressive splitting off of bases, as a consequence of which more and more sugar aldehydes are made available for the reaction, which would lead one to expect a maximum when all the purine-desoxyribose linkages have been broken. But the decrease in color after the maximum intensity of stain is reached is quite unexpected. Bauer offered two possible explanations for the latter phenomenon: first, it might be due to a continued decomposition of that part of the nucleic acid molecule remaining after the purine bases have been split off; or second, it

might be due to the destruction of the protein-nucleic acid linkage allowing the latter to diffuse from the chromatin. Hillary subsequently showed that the decrease in intensity could not be due to a chemical degradation of the aldehyde or color-producing groups in the sugar, because a solution of thymus nucleic acid in a test tube is still capable of reaction to produce color after eight hours of hydrolysis. Furthermore, Hillary hydrolyzed nucleic acid agar blocks from five to thirty minutes, and after testing both block and hydrolyzing medium with the Schiff reagent found that the staining capacity of the latter was roughly the reciprocal of that in the agar blocks. This last experiment suggests that one result of prolonged hydrolysis is to change the nucleic acid into a more readily diffusible form.

In order to reach a clearer understanding of what might be happening to the nucleic acid molecule as time of hydrolysis is increased, Professor Arthur W. Pollister suggested that an attempt be made to follow the fate of the molecule by separate photometric determination of each of the three constituents of the individual mononucleotides. Cartilage cells from the head region of frog tadpoles were selected as especially suitable for these measurements, since in these cells the nuclei are nearly perfect spheres and the cytoplasm contains little ultra-violet absorbing material. The diameter of these nuclei is about seven microns. The cartilage was fixed in Carnoy's acetic alcohol (1:3), and sections were cut at ten microns. Only whole nuclei were measured, and corrections were made for the cytoplasmic absorption and for the non-specific light loss as determined in appropriate blanks. The fate of the nucleic acid bases was followed by means of ultra-violet absorption, that of the desoxypentose by measuring the amount of dye produced by the Feulgen reaction performed in the manner described by Stowell,⁴ and that of the phosphoric acid by overstaining with methyl green and removing most of the dye not chemically combined, by prolonged washing in absolute isopropyl alcohol.

Bauer constructed curves based only upon visual estimates of Feulgen dye intensity; and the similar curve of Fig. 1, which is based upon actual photometric measurements of absorption by the dye in nuclei, agrees fully with his semi-quantitative observations. There is a peak at 12 minutes which is followed by a decline until at 24 minutes the extinction value is barely measurable.

The change in concentration of purine and pyrimidine bases throughout hydrolysis is shown by the curve based on the ultra-violet absorption. Feulgen⁵ found that mild acid hydrolysis, like that in the nucleal reaction, readily breaks the purine-sugar linkage, while by contrast, the pyrimidine-sugar linkage is broken only after much more drastic treatment. It has been recently shown that most, perhaps all, desoxypentose nucleic acids contain equal amounts of purines and pyrimidines (Mirsky and Pollister⁶); consequently, in the Feulgen reaction the time when the ultra-violet ex-

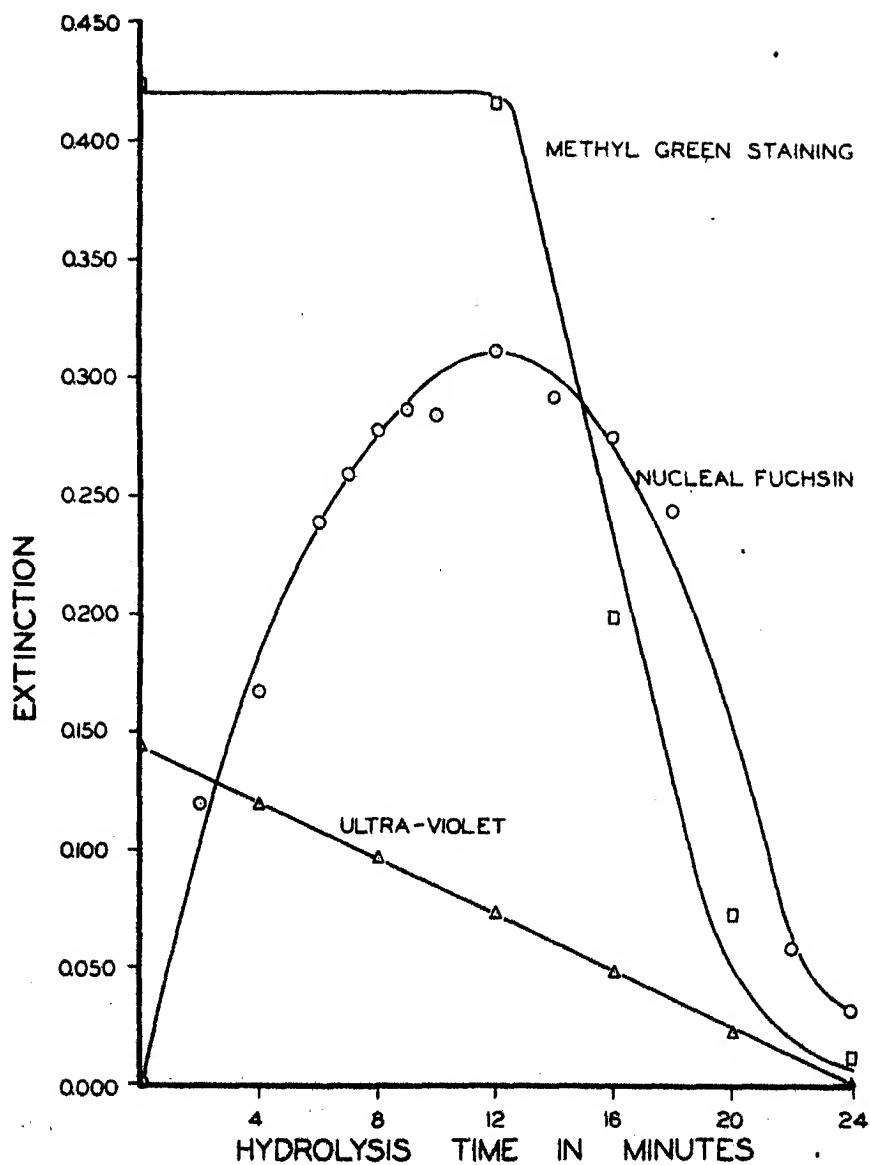


FIGURE 1

Absorption measurements of cartilage cell nuclei from the head region of frog tadpoles at various times of hydrolysis. Each point represents the statistical mean of from 5 to 25 nuclei depending upon the number of nuclei necessary to show a significant difference between consecutive points at a 5% level of significance.

tion has reached a value one-half that of the unhydrolyzed nuclei would be expected to correspond to that point at which all the purines have been removed by hydrolysis. The extinction for unhydrolyzed nuclei was found to be 0.140. A measured value of 0.070, one-half that of the unhydrolyzed nuclei, was found at 12 minutes hydrolysis. As we saw above, this was the moment when the amount of regenerated fuchsin dye was at its maximum. It appears likely therefore, that for the first 12 minutes' hydrolysis the Feulgen reaction is proceeding in the accepted manner, i.e., by the progressive splitting off of purines producing in the desoxypentose more and more free aldehyde groups which become available for restoring color to the decolorized fuchsin.

Turning to a consideration of the effect of longer hydrolysis, it can be seen from the ultra-violet absorption curve that beyond 12 minutes the amount of purine and pyrimidine decreases steadily. Finally after 24 minutes' hydrolysis, at the time when we have seen that the Feulgen regenerated dye indicated practically no reacting sugar, the bases likewise are no longer present.

The original nucleic acid of the nuclei contained, presumably, one phosphoric acid radical for each desoxypentose and base residue. The methyl green curve follows the history of this component during the course of the standard hydrochloric acid treatment. At the end of 12 minutes' hydrolysis the nuclei contain the full amount of phosphoric acid. Thus, during a period when up to one-half the base content is being removed from the nucleic acid, and while the Feulgen reaction with the desoxypentose is consequently increasing to a maximum, the third component of the nucleic acid has remained unchanged. This is strong evidence that, *during a hydrolysis which is optimal for production of the Feulgen nuclear color, the change in the nucleic acid molecule involves only the splitting off of purine bases*, as originally postulated by Feulgen. The phosphoric acid curve likewise sheds most interesting light on the effect of longer hydrolysis. Beginning at 12 minutes the phosphoric acid decreases rapidly and at 24 minutes the amount of methyl green bound by the nuclei is barely measurable. Taken with the evidence from fuchsin and ultra-violet absorption, this shows conclusively that the events from 12 minutes on are very different from those during the earlier part of the hydrolysis; for the *overhydrolysis leads to the destruction or loss from the nuclei of all three components of the nucleic acid molecule*. In the light of Hillary's experiments (see above) it is probable that the latter is the correct explanation; the overhydrolysis has altered the nucleic acid so that it freely diffuses out of the chromatin. It would seem that this very likely involves some depolymerization. Another process that may be expected to be taking place is a breaking of the nucleic acid-histone salt linkage; for Carnoy's reagent has been found by Pollister and Ris to be unique among cytological fixing fluids in that it preserves the nuclear histones in such a

form that they are readily removable by chemical means (e.g., by solution in the divalent mercury-sulfuric acid Millon reagent).

This brings us to the question of the relation of fixation to the Feulgen reaction, which was exhaustively studied by Bauer. He found sharp-peaked nuclear reaction curves, like that above, after fixation with such fluids as Carnoy and acetic sublimate. A similar curve has also been found for a trichloroacetic sublimate fixing fluid (Pollister unpublished). By contrast, Bauer found that when tissue is fixed in fluids containing chromic acid (such as Allen's Bouin, San Felice, Flemming, Flemming-Heitz, and Champy) instead of a sharply restricted moment of optimal hydrolysis there is a long optimal period during which there is no change in stain intensity; although very prolonged hydrolysis eventually does lead to a drop in stain intensity. It is interesting that the fixing fluids which Bauer found to give sharp-peaked curves upon hydrolysis are those which fix tissue in such a way that all, or nearly all, the histone is readily removable; while from chromic acid fixed material the same treatment removes little or none of the protein (Pollister unpublished). Experiments are now being performed to measure the amount of histone at different stages of hydrolysis.

A compelling reason for undertaking the present study was to examine the possibility of using the Feulgen reaction for quantitative determination of desoxypentose nucleic acid in a cytological preparation. The following considerations indicate that, under proper conditions, the intensity of the Feulgen reaction is an accurate measure of the amount of desoxypentose nucleic acid in a nucleus. The stain intensity at the peak is fairly accurately reproducible for one type of tissue, and the extinction value at the peak after chromic acid fixation seems to be close to that after Carnoy's fluid. As we have seen above, this peak probably represents a moment when all the purines have been removed. From the extinction value of the optimal Feulgen reaction, one can readily compute the amount of dye in an average nucleus (standard value for regenerated fuchsin, 4.4×10^{-6} gram per cc., 1 cm. path, extinction at $530 m\mu$, 0.745). It is Wieland and Scheuing's⁷ view of the nature of the Schiff reaction, that two parts of each decolorized fuchsin molecule react with two aldehyde groups to restore the color. Hence, if it is assumed that the color in the nucleus after optimum hydrolysis has resulted from the reaction of one dye molecule with two desoxypentose residues to which a purine was originally attached, the total amount of nucleic acid in an average nucleus can be computed. A wholly independent computation of nucleic acid in a cartilage cell nucleus may be made from the extinction, near the ultra-violet absorption peak, of nucleic acid in an unhydrolyzed preparation. By calculation from ultra-violet measurements, the average cartilage nucleus contains 2.44×10^{-12} gram of nucleic acid; from the Feulgen reaction the average nucleus contains 2.39×10^{-12} gram of desoxypentose nucleic acid. A certain amount of justi-

fication for the use of methyl green staining as a measure of nucleic acid phosphoric acid comes from the fact that if one assumes that each of the di-basic methyl green molecules unites with the phosphoric acid of two nucleotides the amount of nucleic acid in the average cartilage nucleus is computed to be 2.44×10^{-12} gram.

It should be added that the above figures are of the same order of magnitude as previous determinations of total desoxypentose nucleic acid in a single nucleus. Pollister and Ris report that the nucleus of the calf thymus lymphocyte contains 1.0×10^{-12} gram, from cytological determination by ultra-violet absorption; while calculation from macrochemical data on the lymphocyte nucleus gave practically the same figure, 1.1×10^{-12} gram. Caspersson⁸ found 11.2×10^{-12} gram of nucleic acid in the large grasshopper leptotene spermatocyte nucleus, by the ultra-violet cytological method. From the macrochemical data of Zittel and O'Dell⁹ the amount of desoxypentose nucleic acid in the nucleus of a single bull spermatozoon can be computed to be 4.6×10^{-12} gram.

Summary.—By photometric measurements the base, sugar and phosphoric acid of the desoxypentose nucleic acid of cartilage nuclei have been followed throughout the standard hydrolysis of the Feulgen nucleal reaction. The results indicate that at optimum hydrolysis time one-half the total base content has been removed; presumably this represents the purines. During post-optimal hydrolysis the whole nucleic acid molecule disappears from the nuclei.

The possibility of using the Feulgen reaction for quantitative determination of desoxypentose nucleic acid is briefly considered.

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DIETARY FACTORS IN THE UTILIZATION OF HOMOCYSTINE*

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Introduction.—It has been known for some time, mainly due to the investigations of du Vigneaud, *et al.*,^{1,2} that homocystine can replace methionine in the diet of the rat provided a methyl donor, such as choline or betaine, is present. The recent work of McKittrick³ has pointed to the same conclusion regarding the chick.

Evidence for the ability of the rat to synthesize methionine from homocystine in the absence of choline, betaine or any other known methyl donor was first presented by Toennies, *et al.*⁴ They found that rats, maintained on a rich pre-experimental diet and then on a depletion diet, lacking choline and sulfur containing amino acids, responded to the addition of choline plus homocystine or to homocystine alone. They discuss the influence of intestinal microorganisms on their results and attempt to minimize this influence by the administration of sulfasuxadine⁵. Even after prolonged sulfonamide treatment their rats showed a positive growth response to homocystine but only in the presence of a liver fraction. This liver fraction was essentially free of methionine and choline. The authors concluded that liver contained a factor or factors making possible the methylation of homocystine from sources other than choline or betaine.

In the course of our investigations of the biochemistry of the ciliated protozoan, *Tetrahymena*, we found that homocystine could replace methionine in its diet.⁶ Choline was present as was a crude growth factor preparation (Factor II) from liver. When Factor II was prepared from plant sources, homocystine alone did not support growth. In addition, subsequent experiments have shown that exogenous choline does not influence the methylation of homocystine but that our Factor II preparation from liver contained the agent described by Toennies and Bennett, while Factor II prepared from plant material (Cerophyl) was devoid of the Toennies and Bennett factor.

The purpose of this report is to present our findings, which support those of Toennies and Bennett, on the occurrence of a factor or factors in liver which allow the methylation of homocystine to methionine in the absence of exogenous choline and betaine. The possibility of unknown microorganisms vitiating the results, always present in work done with vertebrates, is obviated in our work. *Tetrahymena* is grown in pure (bacteria-free) culture and our results reflect the animal's metabolism only. Moreover, it should be remembered that *Tetrahymena* possesses a rather typical

animal biochemical pattern so that comparisons of our results with those on other animals are permissible.

Experimental.—The organism used in this investigation was the ciliated protozoan, *Tetrahymena geleii* W grown in pure (bacteria-free) culture.

In our previous studies on the biochemistry of this organism⁷⁻¹² growth responses were measured by counting the number of ciliates, after appropriate dilutions, in measured volumes of media. The results were expressed as organisms per ml. of fluid. Various attempts to measure growth turbidimetrically had failed, due largely to the tendency of these organisms to stratify before readings could be taken. The Lumetron Colorimeter (Model 400-A) which is of the jewel-bearing type and has an extremely rapid response has proved ideal for this type of work.

TABLE 1
BASE MEDIUM (MINUS METHIONINE)

	$\gamma/\text{ml.}$		$\gamma/\text{ml.}$
L-arginine	125	Biotin (free acid)	0.0005
L-histidine	125	Ca pantothenate	0.10
DL-isoleucine	125	Thiamine HCl	1.00
L-leucine	250	Nicotinamide	0.10
L-lysine	250	Pyridoxine HCl	0.10
DL-phenylalanine	350	Riboflavin	0.10
DL-threonine	125	Pteroylglutamic acid	0.01
L-tryptophane	50	Choline Cl	1.00
DL-valine	125	Hydrolyzed yeast nucleic acid	100.00
DL-serine	250	Factor II (from Cerophyl) 1:5
		Dextrose	1000.00
		M ₉ SO ₄ ·7H ₂ O	100.00
		MgSO ₄	
		K ₂ HPO ₄	100.00
		CaCl ₂ ·2H ₂ O	50.00
		FeCl ₃ ·6H ₂ O	1.25
		MnCl ₂ ·4H ₂ O	0.05
		ZnCl ₂	0.05

All organisms in experimental series were grown in 4-ml. volumes in 14.5 × 125-mm. Pryrex tubes. The tubes were incubated in a slanted position. The increased volume (2-ml. volumes were previously used) was necessary for use in the colorimeter and the slanted position increased the surface area for oxygenation, thereby increasing the yields. All experiments were carried through three serial transplants, readings being taken only on the third transplant. Our method has been to take readings on all third transplant tubes before inoculation, using distilled water as a blank. All readings are made using red filter 650. After inoculation and incubation a second reading is taken. The optical density of the uninoculated tube is subtracted from the optical density obtained after growth has occurred. This procedure is necessary, since the media used

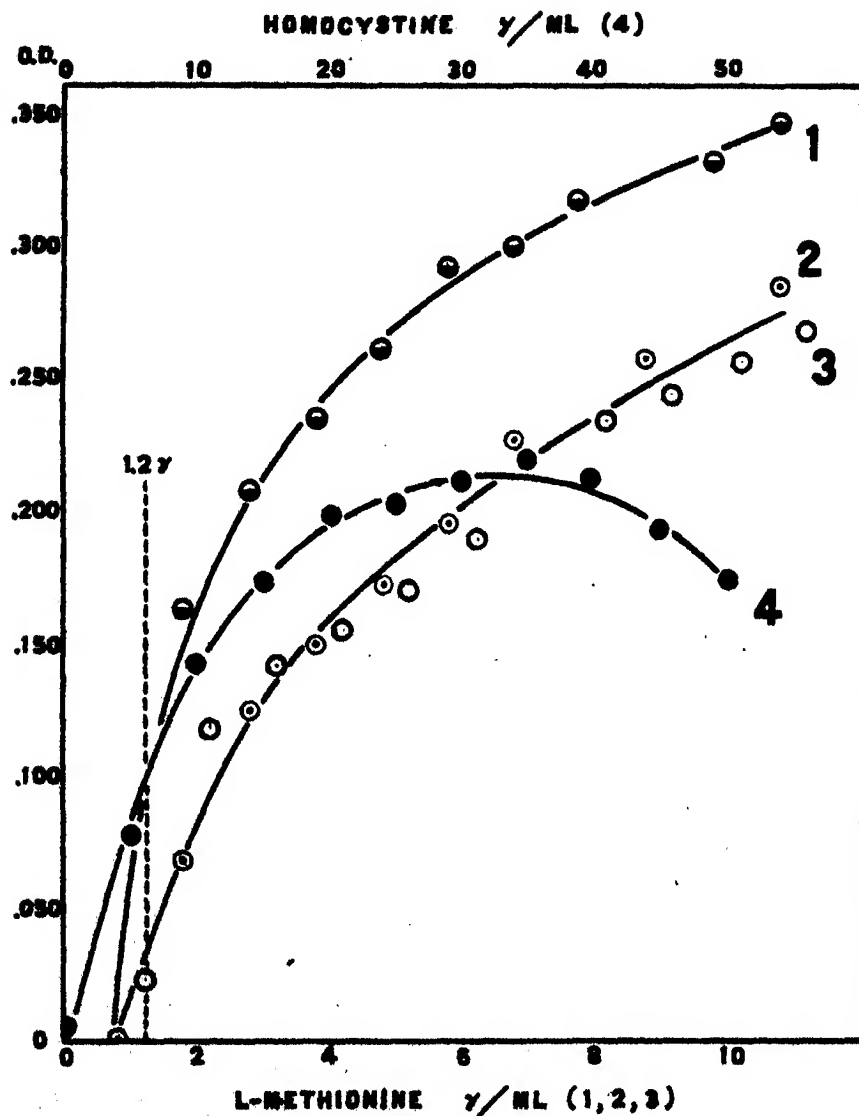


FIGURE 1

Dose response curves. Growth measured in optical density is plotted against amounts in γ per ml. of medium. Curve 1 represents the response to 40 γ of homocystine per ml. and graded amounts of methionine. Curve 2 represents response to methionine only. Curve 3 represents response to methionine in the presence of 120 γ of L.E.L. Curve 4 represents response to homocystine (upper scale) in the presence of 120 γ of L.E.L. (no methionine added). All values from third serial transplants after 6 days incubation.

are colored. With this method tubes need be matched only roughly for size. All readings are taken with the Pyrex label as a position marker. The base medium is given in table 1. This is the same as Medium III as previously described,¹² omitting methionine. Factor II is added as an extract of *Cerophyl*¹³.

The liver fraction used is the same as that employed by Bennett and Toennies⁶ and referred to by them as L.E.L. We are indebted to these authors for a generous supply of this material. In this work we were not concerned with the absolute potency of the active material in the liver fraction, accordingly it was added to the medium and the whole was sterilized by autoclaving for 10 minutes at 15 lbs. pressure. Some destruction of the active material may have occurred. Assays of the L.E.L. and of our Factor II preparation for methionine and choline were carried out microbiologically. *Leuconostoc mesenteroides* P-60 was used for methionine assay¹³ and a cholineless *Neurospora*† mutant¹⁴ for the choline assay. Assays were made on material before and after hydrolysis to preclude failure of detection of bound methionine. It was found that the particular preparation of Factor II used in these experiments contained 2 γ of methionine per ml. The amount used brought in 0.8 γ per ml. of medium. The L.E.L. brought in 0.4 γ per ml. of medium. These amounts were included in calculations when methionine additions were made. In no case was the amount of methionine present lower than 0.8 γ per ml. due to the necessity of adding the growth factor and where L.E.L. was also included there was never less than 1.2 γ per ml. of medium.

Results.—It was found that homocystine could not replace methionine for *Tetrahymena* when the Factor II preparation was obtained from plant material. No growth resulted when methionine was withheld. This result was different from the one previously reported⁶ when Factor II was prepared from Liver fraction L. The addition of varying amounts of L.E.L., however, resulted in growth where methionine was replaced by homocystine in the medium. The minimal concentration of the L.E.L. for moderately good growth (under our conditions) was found to be 120 γ per ml. of medium and this amount was used throughout.

Dose response curves were constructed for homocystine. A typical curve is shown in figure 1 (curve 4). Increased growth was obtained until 40 γ per ml. of homocystine were present. Higher amounts began to show inhibition. As a result of these experiments 40 γ of DL-homocystine per ml. of medium were used routinely.

In view of the fact that 120 γ per ml. of L.E.L. made it possible for the ciliates to utilize homocystine in place of methionine, it was important to determine what effect the L.E.L. would have on growth in the presence of methionine. Accordingly two types of experiments were performed, one to determine the response to methionine alone and the other the response to

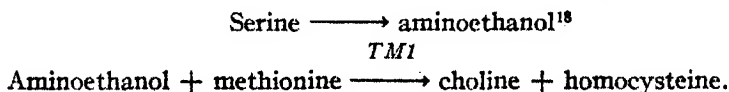
methionine plus 120 γ per ml. of L.E.L. The dose response curves are shown in figure 1 (curve 2 for methionine and curve 3 for methionine plus L.E.L.). It will be noted that the minimum methionine present in curve 2 is 0.8 γ per ml. (from the Factor II) while the minimum for curve 3 is 1.2 γ per ml. (Factor II and L.E.L.). These curves show that the addition of L.E.L. with methionine makes no significant difference.

Cystine must be synthesized, under these conditions, from methionine and the addition of cystine had earlier been shown to spare methionine.⁶ If this synthesis takes place via homocysteine formation as suggested by du Vigneaud¹⁵ then the addition of homocysteine to the medium should likewise spare methionine. This was found to be the case as shown in figure 1 (curve 1). The addition of 40 γ of DL-homocysteine per ml. spared approximately one half of the methionine. This addition made the 1.2 γ of methionine, inevitably present as a minimum when L.E.L. was used, capable of supporting considerable growth (approximately 0.100 on the O.D. scale). It should be noted, however, that 0.8 γ of methionine was insufficient for growth even in the presence of homocysteine. It appears, therefore, that some of the action of the addition of L.E.L. to the homocysteine medium is merely the bringing in of enough methionine to raise the level above the growth threshold. This does not fully account for the resulting growth when L.E.L. and homocysteine replace methionine. Under these conditions, with only 1.2 γ of methionine present, growth is more than double that obtained when L.E.L. is omitted (0.210 on O.D. scale). This difference must be accounted for by the conversion of homocysteine to methionine.

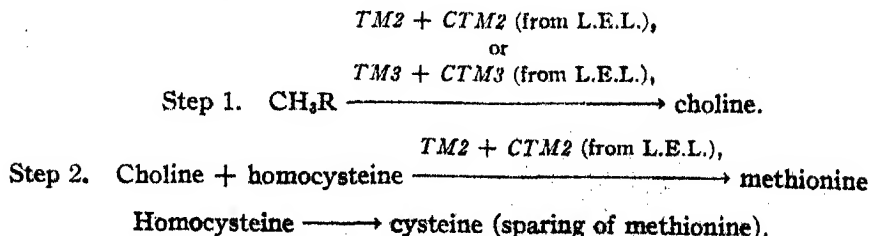
Discussion.—The results outlined above confirm the observations of Toennies and Bennett^{4,5} on the rat and point to an active agent in the liver fraction which in some way makes the conversion of homocysteine to methionine possible. Labile methyl groups in the L.E.L. appear to be precluded in their experiments and in ours as the levels of methionine and choline could not account for enough to transmethylate the amount of homocysteine used† even if all three methyl groups of choline were available. If the statement of McKittrick⁸ regarding the number of methyl groups available from choline is considered, then the amount present is even less nearly adequate. The rat, unlike the chick,^{8,16} can synthesize choline¹⁷ provided methionine is present and we have found rapid synthesis of choline by *Tetrahymena* under like conditions.

Our results differ from those of du Vigneaud, *et al.*^{1,2} and Toennies, *et al.*,^{3,4} regarding choline plus homocysteine. Unlike those workers we found that the addition of choline made no difference in the utilization of homocysteine. Choline plus homocysteine did not permit growth of the ciliates in the absence of adequate methionine or the L.E.L. agent. This fact indicates that the L.E.L. is not supplying a methyl donor but is supplying some agent concerned in the transmethylation process.

As a working hypothesis we make the following proposals to account for the action of the agent in L.E.L. in transmethylation; (I) In *Tetrahymena* the usual source of methyl for the synthesis of choline is methionine and this reaction is made possible by what we will call "transmethylase 1" (TM1). This enzyme appears to require no exogenous co-factor. The reaction may be diagrammed as follows, occurring in two steps:

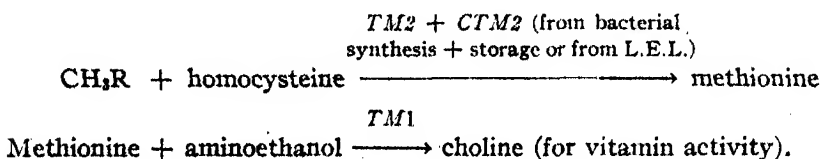


(II) When methionine is replaced by homocystine, methionine is synthesized by the methylation of the latter, which is the reverse of the above reaction. In many cases one and the same enzyme is known to catalyze both a reaction and its reverse. The facts here do not support such activity on the part of TM1. This transmethylation (homocystine to methionine) requires transmethylase 2 (TM2) which must be activated by an exogenous co-factor (CTM2) which the animal organism is incapable of synthesizing and therefore must obtain from its environment. This co-factor appears to be stored in the liver of mammals, presumably concentrated there from the food or from the products of microsynthesis by intestinal bacteria. It appears to be absent or in low concentrations in higher plants. Therefore, only when CTM2 (from L.E.L., in this case) is added with homocystine, is TM2 activated for the required transmethylation to methionine. (III) To account for the source of the methyl groups for this reaction one must assume the synthesis or mobilization of methyl from some unknown precursor (CH_3R), probably first to form choline and then to form methionine via homocystine. (IV) The transmethylation of aminoethanol by CH_3R to form choline is presumably controlled by a transmethylating enzyme also and there is no evidence to indicate whether it is TM2 or a different one (TM3). It cannot be TM1 because no growth of the ciliate results in the presence of adequate choline and homocystine but no L.E.L. If it is a different enzyme from TM2 then it requires a different co-factor (CTM3) and this co-factor is also found in liver. This last assumption will become clear from the discussion of the results on the rat. The above reactions may be diagrammed as follows:



In the rat ¹⁻⁵ step 2 seems to proceed without any exogenous co-factor but this can be explained on the basis of bacterial synthesis, storage or both. Both of these sources are denied *Tetrahymena* so step 2 only proceeds when CTM2 is added. In the rat step 1 is blocked by either too low a concentration of CTM2 for both steps or, if a different co-factor is required (CTM3), then its amount is inadequate within the body. In any case *Tetrahymena*, needing the co-factor for step 2, has a supply provided for step 1 by the addition of L.E.L.

The possibility of the direct methylation of homocysteine from CH₃R to form methionine should perhaps be considered. We know from the work of Simmonds, *et al.*,¹⁹ that in the presence of added choline labeled methyl from choline does appear in the methionine. This need not be the only route of methionine formation, especially when choline, as a vitamin, is being synthesized. This alternative may be diagrammed as follows:



Recently, Horowitz²⁰ has presented evidence to show that the synthesis of methionine by *Neurospora* proceeds from cysteine and each step is controlled by one gene. One mutant failed to grow on homocysteine but did grow on methionine. It would be interesting and important to determine whether this failure to transmethylate is due to a failure on the part of the mutant to synthesize the necessary co-transmethylase. This could be determined by the addition of the active agent from L.E.L. The fact that none of the *Neurospora* mutants could utilize homocysteine and all but the one mentioned above could grow on homocysteine is apart from the transmethylation problem, and concerns only the inability of the mold to reduce homocysteine.¹

The various parts of the above hypothesis are subject to test. Plans are made for the concentration and possible identification of the factor or factors in the liver extract. Factor II preparations entirely free of methionine and choline can be prepared so that the possible effects of traces of these substances can be determined. As concentration of the active agent or agents proceeds tests can be made to determine whether or not more than one co-factor exists which will indicate the relationships between the enzymes controlling steps 1 and 2.

Summary.—1. Homocysteine can replace methionine in the diet of *Tetrahymena* provided some factor or factors found in liver are present.

2. The liver factor appears not to be a methyl donor as is indicated by

the failure of methionine replacement by homocystine in the presence of choline.

3. The liver factor can best be described as a co-transmethylase.

4. A discussion of the mechanism or mechanisms of transmethylation in animals is given.

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† The cholineless *Neurospora* used in this investigation was obtained from Dr. E. L. Tatum of Yale University.

‡ Betaine was absent from L.E.L. according to Bennett and Toennies.⁵

§ Since this manuscript went to press, we have tested strain 38706 (the methionineless mutant of *Neurospora crassa*, referred to as Me-1 by Horowitz²⁰). Several other methionineless strains of *Neurospora* from the Yale collection (6073A, 4195a, 6279a, 4195A(3)) were also tested. All were found to be incapable of utilizing homocystine, even in the presence of L.E.L. All responded to methionine. This indicates that the genetic block occurs in the synthesis of the transmethylating enzyme and not in the synthesis of the cofactor. We wish to thank Dr. N. H. Horowitz for strain 38706 and Drs. E. L. Tatum and S. Simmonds for the other strains and for the sample of homocystine used.

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LINKAGE AMONG GENES CONTROLLING INHIBITION OF LYSIS
IN A BACTERIAL VIRUS*

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Experiments in which bacterial cells are simultaneously infected with two different viruses of the T2 serological group have suggested that both can grow in the same cell,¹ and that interaction between them produces new genetic types of virus.^{2, 3} Further experiments, in which the mixed clones of virus coming from single bacterial cells were examined, have confirmed these inferences, and indicate that the new types arise only in those bacteria in which both viruses succeed in growing freely. These experiments are being continued in an effort to learn something about the mechanism of genetic interaction, and will not be reported in further detail at this time. In this paper we wish to describe a new series of mutations in the bacterial virus T2H,¹ discovered in connection with the experiments mentioned above, which point to the operation in this virus of a system of genetic linkage.

The virus T2H, like all its relatives, exists in the form of a lysis-inhibiting wild type (r^+) and an r (for rapidly lysing) mutant phenotype, which has lost the lysis-inhibiting character.¹ The principal new finding to be reported here is that all the independently arising r mutants of T2H that we have examined are genetically different. The basic observations may be summarized as follows.

A genetic difference between two r mutants is shown when bacteria infected with both liberate viral progeny containing a measurable proportion of lysis-inhibiting types, whereas bacteria infected with the same number of particles of either mutant alone yield only r viral progeny. The experimental procedure used is the one-step growth experiment of Delbrück and Luria, with mixed multiple infection, carried out as described earlier.¹ For simplicity of language, we shall speak of this experiment as a cross, described as heterologous when new types appear among the yield of virus, and homologous when only the parent types are recovered.

Certain details of the method require mention. First of all, one examines by it not the progeny of a genetic cross strictly speaking, but simply a mixed population of virus growing in the same community. The crosses reported in this paper were so arranged that the yield of virus examined came from about 40,000 mixedly infected bacteria. Variations in yields from individual bacteria⁴ are thus left out of account. Care was taken to ensure adequate and equal multiplicity of infection, with respect both to titers of

stocks used and to adsorption to bacteria. Fortunately, all mutants were found to be equally well adsorbed in sensitive tests with admixed wild type (table 2). The effect of unequal multiplicity on the yield of segregants was also tested. The largest proportion of wild type resulted from equal multiplicity of infection with the two parent types, but the yield was not markedly affected by variations between 1 : 2 and 2 : 1. Variations in total multiplicity above about 3 of each type per bacterium had no measurable effect on the result. A multiplicity of about 5 of each type per bacterium was habitually used. Attention was also paid to the total yield of virus in crosses. In parallel experiments, the yields seldom differed by more than 15 per cent, and fell between 300 and 600 per bacterium, depending in part on variations in the nutrient broth. Because of these variations, difficult to control, important comparative results were always checked by parallel experiments.

The *r* mutants so far examined, among which 15 have been named *r*1, *r*2, etc., in order of isolation, are all identical with respect to type of plaque. Their behavior in intercrosses places them in two clearly defined classes, called A and B. Crosses between any pair belonging to the two classes yield about 15 per cent of wild type among the total yield of virus, no significant differences having been established. Crosses within classes yield proportions of wild type characteristic for each pair, falling between 0.5 and 8 per cent. The first eleven *r* mutants to be examined were intercrossed in all combinations, yielding only one example (*r*1) of class A, and no homologous pairs. Six others were examined less completely in a search for a second member of class A; all proved to be class B, and at least two differed from all the others. The second member of class A was finally obtained from an *r* mutant of the distinct virus T4, by crossing with T2H.² This mutant is called *r*14. Three examples of class B were discarded without numbering.

The results of the quantitative intercrosses, summarized in table 1, present three significant features. (1) The amounts of wild type appearing in the three possible crosses among sets of three mutants of class B (e.g., *r*2, *r*5 and *r*7) are approximately additive, but generally fall noticeably short, so that no linear order valid for several loci can be discerned. (2) The results of intercrosses among sets of four mutants (e.g., *r*2, *r*4, *r*5 and *r*7) cannot be interpreted in terms of four independent transfer frequencies, but indicate some type of linkage. (3) If linkage is assumed, the classes A and B are probably to be regarded as independent linkage groups. However, the fact that *r*1 of class A gives equal amounts of wild type with all members of class B is not adequate proof of this independence, for the same thing is seen at a lower level in the crosses between *r*13 and the other members of class B.

The different *r* mutants also differ with respect to rate of spontaneous

TABLE 1
PER CENT OF WILD TYPE IN VIRUS YIELDS FROM BACTERIA INFECTED WITH PAIRS OF *r* MUTANTS

CLASS A		CLASS B								
<i>r</i> 1	<i>r</i> 14	<i>r</i> 13	<i>r</i> 2	<i>r</i> 4	<i>r</i> 8	<i>r</i> 9	<i>r</i> 3	<i>r</i> 5	<i>r</i> 7	<i>r</i> 6
<i>r</i> 1	0.5	14	17	16	(15)	(13)	14	15	15	15
	<i>r</i> 14	(13)	X	X	X	X	X	X	15	X
		<i>r</i> 13	6	6	(7)	X	(7)	(6)	7	8
			<i>r</i> 2	1.1	1.7	1.5	2.0	3.9	3.0	2.4
				<i>r</i> 4	1.1	(1.5)	2.6	2.9	3.5	3.2
					<i>r</i> 8	(0.8)	(2.1)	(2.3)	(2.3)	2.7
						<i>r</i> 9	(0.9)	(1.8)	(2.2)	(2.4)
							<i>r</i> 3	(1.2)	(1.0)	1.4
								<i>r</i> 5	0.8	(1.1)
									<i>r</i> 7	(0.5)

The crosses indicated by X have not been made. Results in parentheses are from single experiments; the others are averages of several. Self-crosses yield less than 0.1% wild type, usually none.

reversion to lysis-inhibitor during serial passage of the virus.¹ In two or more independent tests starting from different single plaques of each of several *r* mutants, the number of passages required to yield lysates containing 10 to 20 per cent of lysis-inhibiting virus varied as follows: for *r*9, two or three; for *r*5, *r*6, *r*11, five; for *r*2, *r*3, *r*4, *r*8, *r*10, *r*14, six or seven; for *r*7, *r*13, eight; for *r*1, ten; and for *r*15, about twenty-two. The lysis-inhibitor recovered from *r*2, *r*13, and *r*15 differed from authentic wild type in appearance of plaques, being of intermediate character between *r* and wild type. On further serial passage, variants appeared which resembled wild type more closely. Both types of lysis-inhibiting virus obtained from these three mutants were genetically different from wild type, for when either was crossed with wild type, excessive numbers of *r* mutants were found among the progeny. This was true of seven independently arising reversions from one or another of the mutants named. Presumably the lysis inhibiting virus selected in these cases arose by suppressor mutations, rather than by back mutation at the respective *r* loci. On the other hand, lysis-inhibiting viruses recovered from each of the 11 remaining *r* mutants mentioned above were indistinguishable from wild type both phenotypically and in back cross. We have not, however, done the careful experiments which might reveal suppressor mutations closely linked to an *r* locus.

Two different *r* loci were found to mutate independently in a double-mutant virus obtained as described in the following section of this paper. In four independent serial passages starting from different single plaques of the double mutant *r*1*r*5, the reversion passed through the more stable type *r*1 in each case. This suggests that the difference in the rate of reversion of the two single mutants is due to differences in stability of loci, rather than to

different selection rates among mutants growing in competition with wild type. Direct estimations of selection rates have not been made, however.

In contrast with the spontaneous reversions, lysis-inhibiting virus arising in crosses between *r* mutants is always homologous with wild type. Tests of this point included segregants from the cross *r*2 × *r*13, two mutants whose spontaneous reversions are heterologous with wild type. There is, furthermore, no correlation between the individual stability of two *r* mutants and the amount of wild type they throw in crosses.

A few of the mutants, including two lines each of *r*2 and *r*13 mentioned above, were carried through the transformations *r* → *hr* → *r* by crossing with a host-range mutant (*h*), followed by a cross with wild type. The resulting stocks showed the same behavior in crosses as the originals. It is unlikely, therefore, that these are multiple-locus mutants.

In order to compare the ability of the different *r* mutants to grow competitively in the bacterial cells, mixed infections with *r* mutant and wild type were made. In only two cases did the proportion of wild type in the

TABLE 2
COMPETITION BETWEEN *r* MUTANTS AND WILD TYPE IN MIXED INFECTION

MUTANT	% WILD TYPE			MUTANT	% WILD TYPE		
	INPUT	ADSORBED	YIELD		INPUT	ADSORBED	YIELD
<i>r</i> 1	54	59	53	<i>r</i> 4 <i>r</i> 7	52	55	53
<i>r</i> 5	50	57	54	<i>r</i> 2	68	77	64
<i>r</i> 1 <i>r</i> 5	53	55	37	<i>r</i> 3	58	58	60
<i>r</i> 13	51	48	60	<i>r</i> 6	60	60	57
<i>r</i> 4	60	64	65	<i>r</i> 2 <i>r</i> 3	63	70	67
<i>r</i> 7	59	54	61	<i>r</i> 2 <i>r</i> 6	60	56	64
<i>r</i> 4 <i>r</i> 7 <i>r</i> 13	59	54	54	<i>r</i> 3 <i>r</i> 6	66	71	69

yield differ appreciably from the proportion in the infecting mixture, or from the proportion in the fraction adsorbed to bacteria. With the mutant *r*13 the yield contained a proportion of wild type in excess of that adsorbed amounting to about 10 per cent of the total virus (3 experiments). The significance of this is not clear. The other exception is easily explained. The cross between the double mutant *r*1*r*5 and wild type yielded an increased proportion of *r* mutant amounting to about 15 per cent of the total virus (2 experiments), owing to the segregation of the two *r* loci described below. For other multiple mutants studied, all of class B, the loss of wild type was too small to be detected, presumably because of linkage between the loci. The data for individual experiments are summarized in table 2.

Segregation of Loci.—The cross between any two *r* mutants yields two new viral types: wild type and an *r* mutant distinguishable from others only by back cross to the parental types, in which it proves homologous with both. This behavior supports the idea that each mutant represents a modification at a different genetic locus among many controlling inhibition of lysis.

For any pair X and Y , the segregation may be written: $(X^+) \times (+Y) \rightleftharpoons (++) \times (XY)$. In testing this hypothesis, it is necessary to prepare single-plaque stocks from a number of the r progeny of the cross, and test each one by back cross to each of the parent types. Tests of this kind have confirmed the hypothesis in every case. It was also found, within the limits imposed by the small scale of the experiments, that in each cross the two recombination types appeared in equal numbers, and the two parental types in the same proportion with which the bacteria were initially infected. These data are shown in table 3. In one experiment, virus yields from single bacteria⁴ infected with $r4$ and $r7$ were found to contain a small proportion of wild type in nearly every burst, the average being about 4 per cent. From one of the yields, tests of a number of r progeny revealed the three additional viral types expected.

TABLE 3
SEGREGATION OF r LOCI IN INTERCROSSES

CROSS	WILD TYPE (% OF YIELD)	DISTRIBUTION OF TYPES AMONG r PROGENY			
$r1 \times r5$	15	4 $r1$	4 $r5$	2 $r1r5$	
$r1 \times r7$	15	9 $r1$	5 $r7$	6 $r1r7$	
$r1 \times r13$	15	5 $r1$	3 $r13$	4 $r1r13$	
$r4 \times r13$	6	12 $r4$	17 $r13$	1 $r4r13$	
$r7 \times r13$	7	7 $r7$	7 $r13$	1 $r7r13$	
$r2 \times r3$	3.3	36 $r2$	47 $r3$	3 $r2r3$	
$r2 \times r6$	3.4	33 $r2$	41 $r6$	1 $r2r6$	
$r3 \times r6$	1.9	63 $r3$	23 $r6$	1 $r3r6$	
$++ \times r1r5$	37	2 $r1$	2 $r5$	6 $r1r5$	
$r1r5 \times r1r13$	0.0	13 $r1r5$	13 $r1r13$	2 $r1r5r13$	2 $r1$
$r4r7 \times r7r13$	0.0	12 $r4r7$	15 $r7r13$	3 $r4r7r13$	0 $r7$

The equality of numbers of the two recombination progeny, only suggested by the data of table 3, has been confirmed on a statistically adequate scale for crosses between host-range and r mutants to be reported elsewhere.

Multiple Factor Tests.—In an effort to learn something about the nature of the linkage, multiple factor crosses have been made. These are of three types.

The first type consists of three factor crosses involving the loci $r1$, $r4$, and $r7$, the two latter being rather closely linked but probably independent of the first. If this is correct, one would expect the factor $r1$ to distribute itself equally between the two types of virus resulting from the exchange of $r4$ with respect to $r7$. The effect should be an equal segregation of wild type from the crosses $r1r4 \times r7$ and $r1r7 \times r4$, amounting in either case to about half that segregating in the cross $r4 \times r7$. For the cross $r1 \times r4r7$, the yield of wild type should be nearly as large as that for $r1 \times r7$, owing to the linkage. The data shown in table 4 confirm these expectations very well.

TABLE 4
SEGREGATION OF WILD TYPE IN MULTIPLE FACTOR INTERCROSSES

CROSS	% WILD TYPE	CROSS	% WILD TYPE
$r1r4 \times r7$	1.4 (3)	$r7r13 \times r4r13 \times r4r7$	0.16 (2)
$r1r7 \times r4$	1.4 (3)	$r4 \times r7 \times r13$	7.3 (1)
$r1 \times r4r7$	11 (2)	$r2r3 \times r2r6 \times r3r6$	0.02 (7)
$r1 \times r7$	15 (4)	$r2 \times r3 \times r6$	3.0 (2)
$r4r13 \times r7$	1.8 (4)	$r2r3 \times r6$	0.8 (7)
$r7r13 \times r4$	0.7 (3)	$r2r6 \times r3$	0.3 (6)
$r4r7 \times r13$	5.0 (3)	$r3r6 \times r2$	1.4 (5)
$r4 \times r13$	6.3 (5)	$r2 \times r3$	2.0 (6)
$r7 \times r13$	7.0 (8)	$r2 \times r6$	2.4 (6)
$r4 \times r7$	3.5 (9)	$r3 \times r6$	1.4 (7)

The results shown are averages of the number of experiments indicated in parentheses. The 22 measurements, including all crosses $r4 \times r13$, $r7 \times r13$ and $r4 \times r7$ made during several months, show a coefficient of variation of 20%.

The second type of cross corresponds to the conventional three factor test for linkage order. We have used two sets of three factors all belonging to the class B. The sets include ($r2$, $r3$ and $r6$) and ($r13$, $r4$ and $r7$), the former being more closely linked than the latter. The data for these crosses, also included in table 4, tend to place the factors in the order listed above, though the correspondence with expectations for random crossing over between linear structures is rather poor. The data for $r2$, $r3$ and $r6$, particularly, are compatible with the possibility that a crossover in one region tends to be accompanied by a second in the adjacent region.

The third type of cross, suggested by analogous experiments with bacteria,⁵ was tried for the purpose of estimating the amount of repeated exchange (i.e., exchange between recombination progeny and progeny of the parental types) taking place under the conditions of the experiments. The combinations used were such that segregation of wild type would require the interaction (simultaneous or successive) of three different mutants. The sets chosen were ($r4r13$, $r7r13$, $r4r7$) and ($r2r3$, $r2r6$, $r3r6$). In different experiments, multiplicities of about three and five per bacterium of each of the three mutants of the set were tested, with similar results. The data for these crosses, and for the corresponding sets of three single mutants, are shown in table 4. It will be seen that repeated exchanges can be detected by this method; that they are less frequent for the more closely linked factors; and that their number is too small to be of quantitative importance in the linkage tests already described.

It should be noted that repeated exchange of the type detected above should not have any effect in two factor crosses. Repeated exchange between sister recombination progeny, if such arise, presents a more serious difficulty. Until effects from this cause can be evaluated, no great quantitative significance can be attached to the results of intercrosses.

Discussion.—Differences among the r mutants described in this paper indicate that there are an indefinite number of distinct loci controlling lysis inhibition in the bacterial virus T2H. The suggestion from the genetic data is that the mechanism of lysis-inhibition⁶ may be exceedingly complex. On the other hand, the fact that identical mutants are not encountered shows that the different r mutations occur with similar frequency. This remarkable circumstance might be interpreted as an indication that the different mutations result from a single type of chemical change occurring at different points in the genetic material of the viral particle. If this were so, the different r mutants might represent alterations of the same biochemical property. This argument must be qualified in view of our ignorance of the number of possible r mutations.

The existence of suppressor mutations noted in this paper, and of intermediate mutant types,³ complicates the system somewhat further.

It is evident that the rather high frequency of r mutations, about 10^{-4} per viral duplication,¹ is at least partly due to the large number of loci involved. It is probably not feasible to measure the stability of any given r locus. For this reason the test of equal rates of r mutation in wild type and in the identical phenotype obtained by reversion from r ,³ is of no value as a test of reversibility of mutation. The test by crossing with wild type, reported in this paper, shows that some reversions are not back mutations, but that most of them probably are.

The situation described for the r mutants does not prevail among viral mutants in general. We have examined six independently arising host-range mutants of the h^+ phenotype,³ which proved to be homologous in intercrosses. This locus can be assigned a position in the linkage class B however, and it is for their usefulness in mapping that the r mutants are likely to prove most valuable.

The linkage system itself we shall discuss only briefly, pending the completion of experiments of another type. It is clear that the classes A and B are independent, or nearly so, and that some kind of linkage exists within the classes. Regarding the linkage structure, some of our data suggest linear arrangement with crossing over, but others are somewhat contradictory. Among difficulties which cannot be discussed very intelligently at present, the necessity for reconciling our data with the results of Luria⁷ should be mentioned. It is probably necessary to add that the linkage system discerned in the virus may yet prove to have little in common with the more familiar linkage systems in cellular organisms.

Summary.—Different r mutants of the virus T2H differ (a) in rate of spontaneous reversion to lysis-inhibiting types; (b) in the kind of reversion they undergo, either to a form genetically like, or to forms unlike, the wild type; (c) in the amount of wild type they throw in intercrosses. With respect to (c) there are two independent linkage groups of distinct r loci.

Analysis of one of these groups by single and multiple factor intercrosses has failed to establish or refute the idea of crossing over between linear structures. The existence of some kind of linkage system conditioning segregation and reassortment of genetic factors among viral particles seems quite clear, but it remains to be learned whether reciprocal, or even material, exchanges are involved.

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A THEORY OF MINIMAX*

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1. Variational analysis, or the theory of critical points, can be examined either analytically or topologically. To furnish the link between these two aspects, it is necessary to establish certain basic properties of the functional in question beyond what is called for in the case of the pure minimum. Lower semi-continuity and compactness are adequate for minima. But precisely this semi-continuity, and not continuity, introduces new questions in the more general case of minimax. A link between the analytic and the topologic is established if the functional can be shown to be *reducible*. This link is completed if it can be shown that the minimax type defined topologically is equal to the minimax type defined analytically. Only when these facts are established is it possible to pass between the domains of analysis and of topology.

In the present note, we shall sketch a theory which does not separate these two basic questions, reducibility and type, but rather makes an organic unity of them (Theorem 3). We discuss a single integral problem where the functional ranges over curves, but it will be clear that the theory is in principle applicable to problems involving surfaces or higher dimensional varieties. Indeed these latter problems are the main motive and

goal of the present work and will be considered in the detailed publication.

2. Consider the variational problem for

$$I[y] = \int_{x_0}^{x_1} f(x, y, y') dx \quad (1)$$

with prescribed end-points $y(x_0) = y_0$, $y(x_1) = y_1$. Let $y = \varphi(x)$ be an extremal passing through these points. We shall suppose that f is sufficiently often differentiable in its arguments x, y, y' and that

$$f_{y'y'}(x, y, p) > 0 \quad (2)$$

for all y in a neighborhood of $\varphi(x)$ and for all p , i.e., we suppose that (1) is a regular variational problem near $y = \varphi(x)$. The second variation of $I[y]$ is

$$\delta^2 I[\eta] = \int_{x_0}^{x_1} [f_{yy}\eta^2 + 2f_{yy'}\eta\eta' + f_{y'y'}\eta'^2] dx \quad (3)$$

where the arguments in the terms involving f are $(x, \varphi(x), \varphi'(x))$ and where

$$\eta(x_0) = \eta(x_1) = 0. \quad (4)$$

The quadratic functional (3) subject to any normalizing condition, say $\int \eta^2 dx = 1$, has certain successive eigenvalues and the corresponding eigenfunctions. Select the first n of them, $\lambda_1, \lambda_2, \dots, \lambda_n$ and $u_1(x), u_2(x), \dots, u_n(x)$, respectively, where n is chosen so that

$$\lambda_{n+1} > 0. \quad (5)$$

The eigenfunctions $u_i(x)$ satisfy

$$(f_{yy}u_i + f_{yy'}u_i') - \frac{d}{dx}(f_{y'y}u_i + f_{y'y'}u_i') - \lambda_i u_i = 0$$

$$u_i(x_0) = u_i(x_1) = 0 \quad (6)$$

and are normalized so that

$$\int_{x_0}^{x_1} u_i(x)u_j(x)dx = \delta_{ij}. \quad (7)$$

With these preliminaries, the following fundamental theorems of the theory can be stated (for Theorems 1, 2 a much weaker hypothesis than (2) can be made).

THEOREM 1. *There is a family of functions*

$$y = \varphi(x; c_1, \dots, c_n) \quad (8)$$

depending on the n parameters c_1, \dots, c_n and defined for sufficiently small absolute values of these parameters, which lie in some neighborhood in the (x, y) plane of $y = \varphi(x)$, which reduces to $y = \varphi(x)$ for $c_1 = \dots = c_n = 0$, and which passes through the end-points $(x_0, y_0), (x_1, y_1)$. For any curve

$y = y(x)$ passing through the end-points and lying in this neighborhood of $y = \varphi(x)$, determine the values of c_1, \dots, c_n by

$$\int_0^1 [y(x) - \varphi(x)] u_i(x) dx = c_i, \quad i = 1, \dots, n \quad (9)$$

Then

$$I[y(x)] \geq I[\varphi(x; c)]. \quad (10)$$

THEOREM 2. For the family $\varphi(x; c)$ we have

$$I[\varphi(x; c)] = I[\varphi(x)] + \sum_{i=1}^n \lambda_i c_i^2 + r \quad (11)$$

where the remainder term r is estimated by

$$\frac{|r|}{c_1^2 + \dots + c_n^2} \rightarrow 0 \text{ as } c_1, \dots, c_n \rightarrow 0$$

uniformly in c_1, \dots, c_n .

THEOREM 3. Make the additional assumption that there are positive numbers A, M such that

$$\left| \frac{f_v(x, y, p)}{p f_{vv}(x, y, p)} \right| \leq M \text{ for all } |p| \geq A \quad (12)$$

and for all (x, y) in a neighborhood of $y = \varphi(x)$. Consider the quantity

$$I(t) = I[\varphi(x; c) + t\{y(x) - \varphi(x; c)\}] \quad (13)$$

as a function of t in $0 \leq t \leq 1$, where c_1, \dots, c_n are determined from (9). Then

$$\frac{dI(t)}{dt} \geq \frac{1}{2} \frac{I(t) - I(0)}{t} \geq 0$$

and

$$I(t) \leq I(0) + t^{1/2} \{I(1) - I(0)\}.$$

3. To prove Theorems 1, 2, consider the problem of minimizing $I[y(x)]$ among curves passing through (x_0, y_0) , (x_1, y_1) and satisfying the side conditions (9) for fixed but small values of c_1, \dots, c_n . The extremals of this new variational problem are solutions of

$$f_v - \frac{d}{dx} f_{v'} - \sum_{i=1}^n \mu_i u_i(x) = 0 \quad (14)$$

where μ_1, \dots, μ_n are constant Lagrange multipliers, and they must also satisfy (9). Let

$$Y(x, b, \mu_1, \dots, \mu_n) \quad (15)$$

be solutions of (14) with initial conditions

$$Y(x_0, b, \mu) = y_0$$

$$Y_x(x_0, b, \mu) = b + \sum_{i=1}^n \frac{\mu_i}{\lambda_i} u_i'(x_0). \quad (16)$$

A particular solution of (14), (16) with $\mu_1 = \dots = \mu_n = 0$ and $b = \varphi'(x_0)$ is $\varphi(x)$.

We establish first that

$$Y_{\mu_i}(x, \varphi'(x_0), 0) = \frac{1}{\lambda_i} u_i(x). \quad (17)$$

Then insert (15) for $y(x)$ in (9) and consider the resulting equations as n equations for the determination of μ_1, \dots, μ_n in terms of b, c_1, \dots, c_n . For $b = \varphi'(x_0), c_1 = \dots = c_n = 0$, a solution of these equations is $\mu_1 = \dots = \mu_n = 0$. By virtue of (17), we prove that the Jacobean of these equations, evaluated for $b = \varphi'(x_0), c_1 = \dots = c_n = 0$, is $\neq 0$. Therefore we can solve the equations for μ_1, \dots, μ_n in terms of b, c_1, \dots, c_n for b sufficiently near $\varphi'(x_0)$ and c_1, \dots, c_n sufficiently small in absolute value. Substituting in (15) gives a function

$$Z(x, b, c_1, \dots, c_n) \quad (18)$$

which is an extremal and satisfies (9).

We establish now that the equation

$$Z(x_1, b, c) = y_1$$

has a unique solution for b in terms of c_1, \dots, c_n for sufficiently small values of these parameters. Substituting in (18) gives the final function

$$y = \varphi(x; c_1, \dots, c_n) \quad (19)$$

which is an extremal, passes through the given points and satisfies (9).

For the extremal (19), the second variation of the new variational problem is

$$\int_{x_0}^{x_1} \{ \bar{f}_{\eta\eta} \eta^2 + 2\bar{f}_{\eta\eta'} \eta \eta' + \bar{f}_{\eta'\eta'} \eta'^2 \} dx \quad (20)$$

with

$$\int_{x_0}^{x_1} \eta u_i(x) dx = 0, \quad i = 1, \dots, n, \quad (21)$$

and (4). The bar over the quantities involving f in (20) indicate that the argument is $(x, \varphi(x; c), \varphi'(x; c))$. The quadratic functional (3) subject to (21) and (4) is positive definite, and has a value $\geq \lambda_{n+1} \int \eta^2 dx$. Since $\varphi(x; c)$ and $\varphi'(x; c)$ are near $\varphi(x), \varphi'(x)$, the second variation (20) subject to (21) and (4) is also positive definite. The positive-definiteness of the second variation implies that the extremal (19) can be imbedded in a

suitable field of extremals. Furthermore, by virtue of the condition (2) and the fact that the side conditions (9) do not involve y' , the Weierstrass E-function for this variational problem is positive. Therefore (19) actually minimizes, and this proves Theorem 1.

Theorem 2 follows from the method of determination of $\varphi(x; c)$. For we prove that

$$\varphi_{c_j}(x; 0, \dots, 0) = u_j(x),$$

and this will yield Theorem 2.

Incidentally in (16) we tacitly assumed that none of the λ_i are zero. If some $\lambda_i = 0$, a suitable modification of (16) can be made.

4. To prove Theorem 3, consider the integral

$$J[\omega] = \int_{x_0}^{x_1} g(x, \omega, \omega') dx \quad (22)$$

where

$$\begin{aligned} g(x, \omega, \omega') = & \omega f_v(x, \varphi(x; c) + \omega, \varphi'(x; c) + \omega') \\ & + \omega' f_{v'}(x, \varphi(x; c) + \omega, \varphi'(x; c) + \omega') \\ & - 1/2 f(x, \varphi(x; c) + \omega, \varphi'(x; c) + \omega') \end{aligned} \quad (23)$$

and where $\omega(x)$ is subject to the side conditions

$$\int_{x_0}^{x_1} \omega u_i(x) dx = 0, \quad i = 1, \dots, n \quad (24)$$

and the end conditions

$$\omega(x_0) = \omega(x_1) = 0. \quad (25)$$

The variational problem for $J[\omega]$ subject to (24), (25), which we shall call problem J , has as Euler equation

$$g_\omega - \frac{d}{dx} g_{\omega'} - \sum_{i=1}^n \mu_i u_i(x) = 0. \quad (26)$$

But for the curve $\omega = 0$, we have

$$g_\omega = 1/2 \bar{f}_v, \quad g_{\omega'} = 1/2 \bar{f}_{v'}$$

where the bar over the f indicates that the argument is $(x, \varphi(x; c), \varphi'(x; c))$. Since $\varphi(x; c)$ satisfies (14) for suitable values of μ_1, \dots, μ_n , it follows that $\omega(x) \equiv 0$ satisfies (26) for these constants μ_1, \dots, μ_n and so

$$\omega(x) \equiv 0 \quad (27)$$

is an extremal for problem J .

Evaluating for the extremal (27), we have

$$g_{\omega'\omega'} = 3/2 \bar{f}_{v'v'} > 0. \quad (28)$$

The second variation of J is likewise $3/2$ times the expression (20), with

side conditions (21). The extremal can therefore be imbedded in a field of extremals for the problem J .

The Weierstrass E-function

$$E = g(x, \omega, \sigma) - g(x, \omega, \omega') - (\sigma - \omega')g_{\omega'}(x, \omega, \omega') \quad (29)$$

for ω, ω' in the field, and σ arbitrary, can be shown to be positive in a sufficiently small neighborhood of (27). For from (28), there is a constant a such that $E > 0$ for $|\sigma| \leq a$ (and $\sigma \neq \omega'$). By virtue of (2) and (12), there is a constant B such that $E > 0$ for $|\sigma| \geq B$. Finally, in the range $a \leq |\sigma| \leq B$ we can make $E > 0$. In all three cases, sufficiently small neighborhoods of (27) in the field must be taken. This establishes the fact that (27) actually minimizes $J[\omega]$ in the final neighborhood selected. Hence

$$J[\omega(x)] \geq -1/2 I[\varphi(x; c)]. \quad (30)$$

Consider the quantity $I(t)$ defined in (13). We have

$$t \frac{dI(t)}{dt} - \frac{1}{2} I(t) = J[\omega] \quad (31)$$

where

$$\omega = t\{y(x) - \varphi(x; c)\}.$$

Inequality (30) yields

$$t \frac{dI(t)}{dt} - \frac{1}{2} I(t) \geq -\frac{1}{2} I(0).$$

This is the first inequality of Theorem 3. The second inequality is an immediate consequence of the first.

5. Theorem 3 can be formulated as a fundamental deformation theorem which establishes the reducibility of $I[y]$ and the characterization of the minimax type of the extremal $y = \varphi(x)$. Select n so that λ_{n+1} is the first positive eigenvalue. There is a neighborhood of $y = \varphi(x)$ such that any curve $y(x)$ in this neighborhood can be deformed into $\varphi(x; c)$ in such a way that the functional $I[y]$ always decreases, and at a rate given in a uniform way in Theorem 3. The end family $\varphi(x; c)$ is an n -parametric family for which Theorem 2 holds. This proves reducibility and at the same time shows, in case none of the λ_i are zero, that the topologic type of minimax is exactly equal to the number of negative eigenvalues of the associated boundary value problem (6).

* Dedicated to Richard Courant on his 60th birthday.

ON ALL OF MERSENNE'S NUMBERS PARTICULARLY M_{193}

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On November 27, 1947, the author finished investigating, by application of the Lucasian sequence 4, 14, 194, 37634, ..., the factorizability of Mersenne's number $M_{193} = 2^{193} - 1 = 1255\ 42034\ 70773\ 36152\ 76715\ 78846\ 41533\ 28322\ 04710\ 88892\ 80690\ 25791$. The 192nd residue had the value 542 45701 25193 90814 13211 43009 56802 04633 04970 79432 42801 51282 which, being non-zero, shows¹ that M_{193} is composite in character. A sufficiently detailed account of the procedure followed and of the underlying basic theorem may be found in the author's first paper² on Mersenne's numbers. Suffice it to record two facts. After the date given above the data of the entire set of original work-strips were checked for the third time with an auxiliary modulus in conformity with the condition $(M_{193}q_k + r_k + 2) \equiv r_{k-1}^2 \pmod{10^7 + 1}$, where q and r denote "quotient" and "remainder," respectively. The approximation to the reciprocal of M_{193} used in the latest work had the value 0. (58 zeros) 79654 59555 66226 13851 44401 98883 85590 27955 52277 59630 93930 37006 37523 90143 41987 11093 10854 40700 94876 19334 41734 25085 25958 34273 96290 7... The check multiplication gave $(M_{193})(1/M_{193})_a = 1 + 1.98 \times 10^{-126}$.

With regard to the 55 numbers of the form $2^p - 1$ (where p is a prime not exceeding 257) considered by Mersenne, the following brief report on recent progress made in their study and the present state of our knowledge may be timely because of its definitive character. In the year 1935 there remained³ six Mersenne numbers which had not been investigated with respect to their prime or composite properties. These corresponded to $p = 157, 167, 193, 199, 227$ and 229 .

In leisure hours the present writer began the investigation of M_{157} in the spring of the year 1944 and finished the entire set with M_{199} on the date given above. These M_p 's were taken up in random order in the vain hope of discovering a prime number greater than $2^{127} - 1$. More specifically the final residues for M_{157} , M_{167} , M_{229} , M_{199} and M_{227} were obtained, respectively, on Aug. 11, 1944, Dec. 2, 1944, Feb. 9, 1946, July 27, 1946, and June 4, 1947. (The manuscript on M_{227} was accepted for publication in the *Bulletin of the American Mathematical Society* on July 17, 1947.) Since all six M_p 's were found to be composite the earlier status of Mersenne's remarks has not been changed but a lacuna in our knowledge has been filled. The next extremely difficult step will consist in the discovery of all of the as yet unknown factors of Mersenne's numbers.

Mersenne said⁴ that the only values of p not greater than 257 which

make M_p prime are 2, 3, 5, 7, 13, 17, 19, 31, 67, 127 and 257. Comparison of this list with the correct data recorded in the top line of the table presented below shows that Mersenne made five mistakes. $p = 67$ and 257 do not yield prime values for M_p , and $p = 61, 89$ and 107 were not included in his list of special primes.

With reference to explicit factoring, attention should be called to a valuable paper⁴ by Professor D. H. Lehmer entitled "On the Factors of $2^n \pm 1$." His investigations on 76 numbers unveiled eleven factors which fall within Mersenne's range. Incidentally two of his new factors confirmed the present writer's final residues for M_{167} and M_{229} .

p	CHARACTER OF M_p
2, 3, 5, 7, 13, 17, 19, 31, 61, 89, 107, 127	Prime
11, 23, 29, 37, 41, 43, 47, 53, 59, 67, 71, 73, 79, 113	Composite and fully factored
151, 163, 173, 179, 181, 223, 233, 239, 251	Two or more prime factors found
83, 97, 131, 167, 191, 197, 211, 229	Only one prime factor known
101, 103, 109, 137, 139, 149, 157, 193, 199, 227, 241, 257	Composite but no factor known

¹ Lehmer, D. H., *Jour. London Math. Soc.*, 9-10, 162-165 (1934-1935).

² Uhler, H. S., *Proc. Nat. Acad. Sci.*, 30, 314-316 (1944).

³ Archibald, R. C., *Scripta Mathematica*, 3, 112-119 (1935).

⁴ Lehmer, D. H., *Bull. Amer. Math. Soc.*, 53, 164-167 (1947).

NEW TYPES OF CONGRUENCES INVOLVING BERNOULLI NUMBERS AND FERMAT'S QUOTIENT

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In another paper¹ the writer gave the relation

$$(mb + k)^n = \sum_{a=1}^r \binom{r}{a} (-1)^{a-1} \frac{S_n(m, k, a)}{a}; \quad r > n; \quad (1)$$

where we define the Bernoulli numbers b_n by means of the recursion formula $(b + 1)^n = b_n$; $n > 1$, the left-hand member being expanded in full and b , substituted for b' , and the left-hand member of (1) is interpreted in the same way;

$$S_n(m, k, a) = \sum_{i=0}^{a-1} (im + k)^n; \quad 0^0 = 1,$$

m and k are any integers with $m \neq 0$. In the present paper we shall employ (1) as well as other known relations to obtain various congruences. The principal results proved seem to be (5), (6b), (7) and (20).

By the well-known Bernoulli summation formula we also have

$$(b + k)^n = nS_{n-1}(k) + b_n, \quad (2)$$

where

$$S_{n-1}(k) = S_{n-1}(1, 0, k).$$

Set in (1), $r = p - 1$ we have, if $n < p - 1$, p being an odd prime,

$$(mb + k)^n = \sum_{a=1}^{p-1} \binom{p-1}{a} (-1)^{a-1} \frac{S_n(m, k, a)}{a}. \quad (3)$$

It is known that

$$\binom{p-1}{a} \equiv (-1)^a \pmod{p}, \quad (3a)$$

so that from (3) we obtain

$$(mb + k)^n \equiv - \sum_{a=1}^{p-1} \frac{S_n(m, k, a)}{a} \pmod{p}. \quad (4)$$

For a particular $a = i$, the corresponding term on the right may be written

$$- \sum_{i=1}^{p-1} \frac{k^n + (k+m)^n + \dots + (k+(i-1)m)^n}{i} = C.$$

Now collect the terms in the right-hand member of (4) involving each particular n th power, we find

$$C = - \sum_{s=0}^{p-2} (k + ms)^n \left(\frac{1}{s+1} + \frac{1}{s+2} + \dots + \frac{1}{p-1} \right).$$

But also

$$1 + \frac{1}{2} + \dots + \frac{1}{p-1} \equiv 1 + 2^{p-2} + \dots + (p-1)^{p-2} \equiv 0 \pmod{p}, \quad (4a)$$

since p is odd. The last result gives

$$- \left(\frac{1}{s+1} + \frac{1}{s+2} + \dots + \frac{1}{p-1} \right) \equiv 1 + \frac{1}{2} + \dots + \frac{1}{s},$$

so that (4) may be written, $n < p - 1$,

$$(mb + k)^n \equiv \sum_{a=1}^{p-2} (ma + k)^n R_a \pmod{p}, \quad (5)$$

where

$$R_a = 1 + \frac{1}{2} + \dots + \frac{1}{a}.$$

For $k = 0$, $m = 1$, this becomes

$$b_n \equiv \sum_{a=1}^{p-2} a^n R_a \pmod{p}. \quad (5a)$$

Also, (5) gives for g any integer, $0 < g < p$, for $mg + k \not\equiv 0 \pmod{p}$,

$$\begin{aligned} & \sum_{n=0}^{p-2} (mg + k)^{-n+p-2} (mb + k)^n \\ & \equiv \sum_{a=1}^{p-1} R_a \sum_{n=0}^{p-2} (ma + k)^n (mg + k)^{p-2-n} \\ & \equiv -(mg + k)^{p-2} R_g + \sum_{\substack{a=1 \\ a \neq g}}^{p-1} R_a \frac{(ma + k)^{p-1} - (mg + k)^{p-1}}{m(a - g)}, \end{aligned}$$

or

$$\sum_{n=0}^{p-2} (mg + k)^{-n+p-2} (mb + k)^n \equiv -(mg + k)^{p-2} R_g, \quad (5b)$$

modulo p , by Fermat's theorem, provided $mg + k \not\equiv 0 \pmod{p}$. For $m = 1$, $k = 0$ this reduces to

$$\sum_{n=0}^{p-2} g^{-n} b_n \equiv -R_g \pmod{p}, \quad (6)$$

which for $g = 1$ becomes²

$$b_0 + b_1 + \dots + b_{p-2} \equiv -1 \pmod{p}. \quad (6a)$$

Taking the relation (5a) for $n = 0, 1, \dots, p - 2$ in turn introducing powers of the integers m , prime to p , similarly to the procedure in the proof of (5b) we obtain

$$\sum_{n=0}^{p-2} g^{-n} m^n b_n \equiv -R_a \pmod{p}, \quad (6b)$$

where $am \equiv g \pmod{p}$; $a < p$.

Now take (5a) again and let $p = 1 + cm$ and put $n, n + c, \dots, n + (m - 1)c$, in turn in this congruence, and add, we obtain if $n < c$

$$\sum_{i=0}^{m-1} b_{n+ic} \equiv m \sum_r r^n R_r \pmod{p}, \quad (7)$$

where r ranges over the distinct solutions in the set $1, 2, \dots, p - 1$ of $x^c \equiv 1 \pmod{p}$. The relation (7) has a type of analogy with the relation³

$$\frac{\sum_r r^n}{p} \equiv -cn \sum_{k=0}^{m-1} \frac{b_{kc+n}}{kc + n} \pmod{p}, \quad (8)$$

with c and n even. Set $\mu_k \equiv k/m \pmod{p}$; $0 < \mu_k < p$. Then

$$\begin{aligned}(mb + k)^n &= m^n \left(b + \frac{k}{m}\right)^n \equiv m^n (b + \mu_k)^n \\ &\equiv m^n (b_n + n(0^{n-1} + 1^{n-1} + \dots + (\mu_k - 1)^{n-1})) \pmod{p},\end{aligned}$$

on applying (2). This gives

$$\frac{(mb + k)^n - m^n b_n}{n} \equiv m^n (0^{n-1} + 1^{n-1} + \dots + (\mu_k - 1)^{n-1}),$$

modulo p . Setting $n = 1, 2, \dots, p-1$, in turn we obtain by adding, since

$$1 + (ma) + (ma)^2 + \dots + (ma)^{p-2} \equiv \frac{(ma)^{p-1} - 1}{ma - 1} \pmod{p},$$

unless $ma - 1 \equiv 0 \pmod{p}$,

$$\sum_{i=1}^{p-1} \frac{(mb + k)^i - m^i b_i}{i} \equiv m \text{ or } 0 \pmod{p}, \quad (9)$$

according as $\mu_1 \geq \mu_k$ or $\mu_1 < \mu_k$. In particular the right-hand member is m if $k = 1$. Using the known relations,

$$S_n(p) \equiv pb_n \pmod{p},$$

$0 < g < p$, the first congruence following involving p terms on the left, we have

$$\begin{aligned}g^{p-2}(1 + 1 + 1 + \dots + 1) &\equiv pb_0 g^{p-2} \pmod{p^2}, \\ g^{p-3}(1 + 2 + \dots + p-1) &\equiv pb_1 g^{p-3} \pmod{p^2}, \\ &\vdots \\ 1^{p-2} + \dots + (p-1)^{p-2} &\equiv pb_{p-2} \pmod{p^2},\end{aligned}$$

and we obtain by addition

$$pg^{p-2} + \sum_{\substack{a=1 \\ a \neq g}}^{p-1} \frac{a^{p-1} - g^{p-1}}{a - g} \equiv -p \frac{R_g}{g} \pmod{p^2},$$

employing (6), and if we write, with $(a, p) = 1$,

$$q(a) = \frac{a^{p-1} - 1}{p},$$

then the above gives

$$g^{p-2} + \sum_{\substack{a=1 \\ a \neq g}}^{p-1} \frac{q(a) - q(g)}{a - g} \equiv -\frac{R_g}{g} \pmod{p}.$$

Now modulo p ,

$$g^{p-2} + \sum_{\substack{a=1 \\ a \neq g}}^{p-1} \frac{q(a)}{a-g} - \sum_{\substack{a=1 \\ a \neq g}}^{p-1} \frac{q(g)}{a-g} \equiv \sum_{\substack{a=1 \\ a \neq g}}^{p-1} \frac{q(a)}{a-g} - q(g)g^{p-2} + g^{p-2}$$

using

$$\sum_{\substack{a=1 \\ a \neq g}}^{p-1} \frac{1}{a-g} \equiv \sum_{a=1}^{p-1} (a-g)^{p-2} \equiv g^{p-2} \pmod{p},$$

hence

$$\frac{1-q(g)}{g} + \sum_{\substack{a=1 \\ a \neq g}}^{p-1} \frac{q(a)}{a-g} \equiv -\frac{R_g}{g} \pmod{p}, \quad (9a)$$

which for $g = 1$ gives

$$\sum_{a=2}^{p-1} \frac{q(a)}{a-1} \equiv -2 \pmod{p}.$$

We now obtain another formula related to (5b). Employing¹ (relation (4))

$$(b(m, k) + m)^{n+1} - b_{n+1}(m, k) = m(n+1)k^{p-2},$$

and setting $n = p-2$, we have after employing (3a),

$$\sum_{i=0}^{p-2} (-1)^i b_i(m, k) m^{p-1-i} \equiv -mk^{p-2},$$

modulo p , where $(mb+k)^i = b_i(m, k)$.

We shall now modify some of the ideas in a certain proof² of (8) to obtain new types of congruences involving the Bernoulli numbers and Fermat's quotient. We have⁴

$$\frac{(1-m^n)b_n}{nm^{n-1}} \equiv \sum_{k=1}^{m-1} \sum_{i=1}^{\lfloor kp/m \rfloor} i^{n-1} \pmod{p}, \quad (10)$$

where $[x]$ is the greatest integer in x , $m > 1$, $n > 1$, $(m, p) = 1$. For $n = 1$ we shall now show that

$$(m-1)b_1 \equiv \sum_{k=1}^{m-1} \sum_{i=1}^{\lfloor kp/r \rfloor} i^{n-1} \pmod{p}. \quad (10a)$$

We have for $(k, m) = 1$, $(m, p) = 1$.

$$pk = r_k + m \left[\frac{pk}{m} \right]; \quad 0 < r_k < m.$$

Set $k = 1, 2, 3, \dots, m-1$ in turn and add we have since $pk_1 \equiv pk_2 \pmod{m}$ only if $k_1 = k_2$ and therefore the r 's range in some order over $1, 2, \dots, m-1$,

$$m \sum_{k=1}^{m-1} \left[\frac{pk}{m} \right] + m \frac{m-1}{2} \equiv 0 \pmod{p},$$

or

$$\sum_{k=1}^{m-1} \left[\frac{pk}{m} \right] \equiv b_1(m-1) \pmod{p}.$$

Now take (10) with (10a), put $n = 1, 2, \dots, p-1$, in turn and add, after multiplying each relation in turn by g^{h-1-n} , we find

$$\frac{m^{p-1}-1}{m^{p-2}} b_{p-1} - \frac{(m-1)}{2} + \sum_{s=2}^{p-2} g^{p-1-s} \frac{1-m^s}{sm^{s-1}} b_s \equiv -g^{p-2} \alpha(g, m) \pmod{p}, \quad (11)$$

since in (10) and (10a) when we add we have on the right

$$\sum_{k=1}^{m-1} \sum_{i=1}^{[pk/m]} (g^{p-2} + ig^{p-3} + i^2 g^{p-4} + \dots + i^{p-2}),$$

or, if $0 < g < p$,

$$\sum_{k=1}^{m-1} \sum_{\substack{i=1 \\ i \neq k}}^{[kp/m]} \frac{i^{p-1} - g^{p-1}}{i - g} + g^{p-2}(p-1)\alpha(g, m),$$

where g^{p-2} occurs $\alpha(g, m)$ times in the right-hand member of (10) and (10a). Now (11) is satisfied identically for $m = 1$. It may also be written

$$(m^{p-1}-1)b_{p-1} - \frac{m-1}{2m} + \sum_{s=2}^{p-2} \frac{m^{p-1-s}-1}{s} g^{p-1-s} b_s \equiv -\frac{g^{p-2}}{m} \alpha(g, m) \pmod{p} \quad (11a)$$

Set $g = 1, m = 1, 2, \dots, p-1$ and add, we have, since for $m < p$, that $\alpha(1, m) = m-1$,

$$(S_{p-1}(p) - p + 1)b_{p-1} + \sum_{s=1}^{p-2} \frac{b_s}{s} \equiv 0 \pmod{p}, \quad (12)$$

since

$$S_0(p) \equiv 0 \pmod{p}$$

for $a < p-1$. Now we can write

$$S_{p-1}(p) \equiv pb_{p-1} \pmod{p^2}. \quad (13)$$

Using (13) and $pb_{p-1} \equiv -1 \pmod{p}$, together with⁶

$$b_{p-1} \equiv 1 + \frac{(p-1)!}{p} \pmod{p},$$

we obtain the known⁶ formula

$$\sum_{s=1}^{p-2} \frac{b_s}{s} \equiv W \pmod{p}, \quad (14)$$

where W is the Wilson quotient

$$\frac{(p-1)! + 1}{p}.$$

On using this in connection with (11a) for $m < p$ we have another known⁶ result

$$-q(m) + \sum_{s=1}^{p-2} \frac{b_s}{sm^s} \equiv W \pmod{p}. \quad (15)$$

Multiplying this last congruence through by a^t gives,⁶ if t is in the range $1, 2, 3, \dots, p-2$,

$$\sum_{a=1}^{p-1} a^t q(a) \equiv -\frac{b_t}{t} \pmod{p}. \quad (16)$$

The relation (15) is an analogous theorem to (6). If $m > 1$, $(m, p) = 1$, p prime and odd, then it is known that⁷

$$\frac{(m^n - 1)b_n}{n} \equiv \sum' (-1)^{n-1} \frac{f_{n-1}(\rho)}{1 - \rho^p} \pmod{p} \quad (17)$$

where

$$f_k(x) = 0^k + 1^k x + 2^k x^2 + \dots + (p-1)^k x^{p-1}$$

with $0^0 = 1$. In this relation set $n = 2, 3, \dots, p-1$, together with the relation

$$0 \equiv r^{p-2} \sum' \frac{\rho + \rho^2 + \dots + \rho^{p-1}}{1 - \rho^p}$$

and add these $p-1$ congruences, we obtain, if $r < p$, the symbol \sum' indicating summation over all the distinct m th roots of unity excepting unity,

$$\sum_{s=1}^{p-1} \sum' \frac{\rho^s (r^{p-2} + r^{p-3} s + \dots + s^{p-2})}{1 - \rho^p}$$

$$\begin{aligned}
&\equiv \frac{r^{p-2}(p-1)r^r}{1-r^p} + \sum' \sum_{\substack{s_1=1 \\ s_1 \neq r}}^{p-1} \frac{s_1^{p-1} - r^{p-1}}{s_1 - r} \div (1-r^p) \\
&\equiv \sum' \frac{r^{p-2}r^r}{r^p - 1},
\end{aligned}$$

modulo p , so we obtain, if we set

$$\beta(m, i) = \sum' \frac{r^i}{(r^p - 1)},$$

for $m > 1$, $0 < r < p$,

$$-q(m) + \sum_{n=2}^{p-2} \frac{1-m^n}{n} r^{p-1-n} b_n \equiv r^{p-2} \beta(m, r) \pmod{p}. \quad (18)$$

But by (15) we also have

$$-\frac{r^{p-2}}{2} - q(r) + \sum_{n=2}^{p-2} \frac{r^{p-1-n} b_n}{n} \equiv W \pmod{p}. \quad (19)$$

Now (18) may be written

$$-q(m) + \sum_{n=2}^{p-2} \frac{r^{p-1-n} b_n}{n} - \sum_{n=2}^{p-2} \frac{m^n r^{p-1-n} b_n}{n} \equiv r^{p-2} \beta(m, r),$$

modulo p , which with (19) gives, if $m > 1$, $0 < r < p$,

$$-q(m) + q(r) + \frac{r^{p-2}}{2} + W - \sum_{n=2}^{p-2} \frac{m^n r^{p-1-n} b_n}{n} \equiv r^{p-2} \beta(m, r), \quad (20)$$

modulo p . For $r = 1$ in particular this takes the form

$$-q(m) + W + \frac{1}{2} - \sum_{n=2}^{p-2} \frac{m^n b_n}{n} \equiv \beta(m, 1). \quad (21)$$

Note that there is a certain type of analogy between (6b) and (20) with b_n corresponding to b_n/n .

¹ *Duke Math. Jour.*, 8, 576 (1941).

² This relation is certainly one of the simplest the author has ever noted concerning the Bernoulli numbers. I have failed to find it in the literature, but do not imagine it could be new. Another proof may be obtained of it by the use of (2) by setting $n = p-1$, and $k = p-1$ therein.

³ These *PROCEEDINGS*, 31, 59, 312-314 (1945).

⁴ *Annals Math.*, 18, 114 (1917); *Duke Math. Jour.*, 3, 571, relation (7) (1937).

⁵ This relation is well known; for a recent proof, cf., Vandiver, *Duke Math. Jour.*, 8, 578 (1941).

⁶ Lerch, *Math. Annalen*, 60, 471-490 (1905); Nielsen, *Oversigt Danske Vidensk. Selsk. Forhandling*, 518-519, 177-180 (1915).

⁷ Included in a result due to Frobenius, *Berlin Sitzungsberichte*, 827 (1910). The latter theorem was generalized by Vandiver, *Trans. Amer. Math. Soc.*, 51, 515 (1942).

CRITICAL POINTS OF HARMONIC FUNCTIONS AS POSITIONS OF EQUILIBRIUM IN A FIELD OF FORCE

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The first mechanical interpretation of the critical points (zeros of the derivative) of a polynomial $p(z)$ is due to Gauss,¹ who showed that the critical points not multiple zeros of $p(z)$ are the positions of equilibrium in the field of force due to unit particles situated at the zeros of $p(z)$, where each particle repels with a force equal to the mass divided by the distance. Bôcher (1904) showed that the zeros of the jacobian of two binary forms and of certain other algebraic invariants can be expressed as the positions of equilibrium in a similar field of force due to positive and negative particles situated at the zeros of the ground forms. Bôcher's results can be applied in the study of the critical points of rational functions.

A given harmonic function can frequently be expressed as the uniform limit of the logarithm of the modulus of a variable polynomial or more general rational function; the critical points of the harmonic function are the limits of those of the rational functions; the present writer (1933 and later) has thus applied the extensive theory of the location of the critical points of a rational function to the study of the critical points of a harmonic function. It is the object of the present note to show that the latter critical points can be conveniently and effectively studied *directly* as positions of equilibrium in a suitably chosen field of force, due to matter spread over Jordan curves or other point sets. The advantages of the present method are a closer analogy between the theory for harmonic functions and that for rational functions, with simpler proofs and stronger theorems, and in addition a systematic method for the enumeration of critical points of harmonic functions. We include also the means for studying various new harmonic functions.

A finite *critical point* of an analytic function $f(z)$ is a zero of the derivative $f'(z)$; the order of the critical point is the order of the zero of $f'(z)$. The point at infinity is a critical point of an analytic function $f(z)$, and of order k , if $z = 0$ is a critical point of $f(1/z)$, and of order k . If $f(z)$ has a pole at infinity, that point is not a critical point; if $f(z)$ is analytic there, the point at infinity is a critical point of order k if and only if $f'(z)$ has a zero there of order $k + 2$. A point $z = x + iy$ is a critical point of a harmonic function $u(x, y)$, and of order k , if it is a critical point of the analytic function $u + iv$, where v is conjugate to u , and of order k .

We shall consider critical points as positions of equilibrium in a field where the force is the conjugate of the derivative $f'(z)$ of an analytic func-

tion $f(z)$. Corresponding to the convention just introduced, we consider the point at infinity to be a position of equilibrium if and only if it is a critical point of $f(z)$.

1. We use the term *Jordan configuration* (of the extended plane) to denote either a continuum composed of a finite number of Jordan arcs or a finite number of such continua, mutually disjoint. Various integrals of potential theory representing a harmonic function $u(x, y)$ in a region R may be taken over the boundary B of R if B is a finite Jordan configuration; ordinarily such an integral is first taken over a level locus L with no multiple points, say $u(x, y) = \text{const}$ interior to R , and on L involves $(\partial u / \partial \nu) ds = -dv$, where $v(x, y)$ is conjugate (locally single valued) to $u(x, y)$ in R , and ν represents interior normal. If $u(x, y)$ is constant on a Jordan configuration J belonging to B , by allowing L to approach J the corresponding integral can perhaps be taken over J itself, on which $v(x, y)$ is continuous. Theorems 1, 5 and 11 below are all proved by this method from the formula

$$u(x, y) = \frac{1}{2\pi} \int_B \left(\log r \frac{\partial u}{\partial \nu} - u \frac{\partial \log r}{\partial \nu} \right) ds, \quad (x, y) \text{ in } R, \quad (1)$$

after making suitable simplifications.

THEOREM 1. *Let the boundary B of an infinite region R be a finite Jordan configuration, and let $G(x, y)$ be Green's function for R with pole at infinity. Then we have*

$$G(x, y) = \int_B \log r \, d\sigma + g, \quad 0 \leq \sigma \leq 1,$$

where $d\sigma = -dH/2\pi$ on B , $\int_B d\sigma = 1$, $H(x, y)$ is conjugate to $G(x, y)$ in R , and g is a constant.

The function

$$H(x, y) = \int_B \arg(z - t) d\sigma(t), \quad z = x + iy,$$

is conjugate to $G(x, y)$ in R , locally single valued, and the function

$$F(z) = G(x, y) + iH(x, y) = \int_B \log(z - t) d\sigma(t) + g$$

is analytic in R .

The critical points of $G(x, y)$ in R are the zeros in R of the analytic function

$$F'(z) = \int_B \frac{d\sigma(t)}{z - t}, \quad z \text{ in } R,$$

and thus are the positions of equilibrium in the field of force due to the spread σ of positive matter on B , where the matter repels with a force proportional to the mass and inversely proportional to the distance.

The last part of Theorem 1 follows by taking the conjugate of $F'(z)$, for

the vector $1/(\bar{z} - \bar{l})$ represents the force at z due to a particle of unit mass at l .

Consideration of the direction of the field of force leads to an analog of Lucas' Theorem:

THEOREM 2. *Under the conditions of Theorem 1, all critical points of $G(x, y)$ in R lie in the smallest convex point set Π containing B . No boundary point z_0 of Π in R is a critical point of $G(x, y)$ unless B lies on a line through z_0 .*

The latter part of Theorem 2 is difficult if not impossible to prove by previously published methods, as is the latter part of Theorem 3 below.

2. If in Theorem 1 the boundary B is symmetric in the axis of reals, the element of mass $d\sigma$ is equal at symmetric points of B . The integral expressing $F'(z)$ can be taken over one half of B , using a suitable convention for any part of B that may lie on the axis, with the integrand

$$\frac{1}{z - t} + \frac{1}{z - \bar{t}}. \quad (2)$$

If z is not real and lies exterior to the *Jensen circle* constructed on the segment (t, \bar{t}) as diameter, the conjugate of (2) is the force at z due to unit particles at t and \bar{t} , and whether or not t is real has a non-zero vertical component directed away from the axis; we have the first part of

THEOREM 3. *Under the conditions of Theorem 1, let B be symmetric in the axis of reals, and let Jensen circles be constructed with diameters the segments joining all pairs of symmetric points of B . Then all critical points of $G(x, y)$ in R lie in the closed interiors of these Jensen circles.*

A critical point z_0 in R not interior to a Jensen circle cannot lie on such a circle unless it lies on all Jensen circles for B , that is to say, unless B lies wholly on the equilateral hyperbola with vertices z_0 and \bar{z}_0 .

The latter part of Theorem 3 follows from the fact that a point z on the Jensen circle for t and \bar{t} , the force represented by the conjugate of (2) is horizontal.

At any point $z_0 = x_0 + iy_0$ of R not a critical point, the force in the field described in Theorem 1 is orthogonal to the locus $G(x, y) = G(x_0, y_0)$; for if we compute dF/dz , where $dz = \epsilon d\nu$ ($|\epsilon| = 1$) is taken in the direction of the normal to this locus in the sense of increasing G , we have $dF/dz = (\partial G/\partial \nu)/\epsilon$, $\partial G/\partial \nu > 0$, $\partial H/\partial \nu = 0$, whence $\arg [F'(z)] = -\arg \epsilon$, so the direction of the force is that of ν ; the conjugate of $F'(z)$ is the gradient of G . This remark obviously applies generally to the field defined by the conjugate of an analytic function $F'(z)$, where $F = G + iH$.

By study of the variation in the direction of the force as z traces a level locus near B , and also traces a suitably chosen auxiliary Jordan curve in R containing K , we study the variation of $\arg [F'(z)]$ on these curves, and obtain

THEOREM 4. *Under the conditions of Theorem 3 let K be a configuration*

consisting of a segment $\alpha \leq z \leq \beta$ plus the closed interiors of all Jensen circles intersecting that segment. Let α and β lie in R and not be critical points of $G(x, y)$. Let K contain k components of B . Then K contains $k - 1$, k , or $k + 1$ critical points of G in R according as the forces at α and β are both directed outward, one outward and one inward, or both directed inward.

3. For forces of the kind described, we prove the

LEMMA. The force at a point P due to a distribution of positive matter in a circular region C not containing P is equal to the force at P due to the same total mass concentrated at a suitably chosen point of C .

A circular region is the closed interior or exterior of a circle, or a half-plane.

This Lemma may be proved by inversion in P ; the force at P due to a particle at Q is in magnitude, direction and sense the vector $Q'P$, where Q' is the inverse of Q ; the proposition is equivalent to the fact that if a distribution of positive mass lies in the closed interior of a given circle, so does its center of gravity. This Lemma enables us to prove many further results, here omitted, from Theorem 1.

The method of proof of Theorem 1 also yields

THEOREM 5. Let R be a region of the extended plane whose boundary B is finite and consists of disjoint Jordan configurations C and D . Let the function $u(x, y)$ be harmonic in R , continuous on $R + B$, zero on C and unity on D . Then in R we have

$$u(x, y) = \int_C \log r \, d\sigma - \int_D \log r \, d\sigma + u_0, \quad \int_C d\sigma = \int_D d\sigma, \quad (3)$$

$d\sigma = -dv/2\pi$ on C , $d\sigma = dv/2\pi$ on D , where u_0 is a constant and $v(x, y)$ is conjugate to $u(x, y)$ in R . We also have

$$v(x, y) = \int_C \arg(z - t) d\sigma - \int_D \arg(z - t) d\sigma, \\ f(z) = u + iv = \int_C \log(z - t) d\sigma - \int_D \log(z - t) d\sigma + u_0.$$

The finite critical points of $u(x, y)$ in R are the finite zeros in R of

$$f'(z) = \int_C \frac{d\sigma}{z - t} - \int_D \frac{d\sigma}{z - t}. \quad (4)$$

Consequently the critical points of $u(x, y)$ in R are precisely the positions of equilibrium in the field of force due to a spread σ of positive matter on C and a spread $-\sigma$ of negative matter on D , where the matter repels with a force proportional to the mass and inversely to the distance.

If R is finite the constant u_0 is zero or unity according as R is separated from the point at infinity by C or D ; if R is infinite, $u_0 = u(\infty)$.

As an analog of a theorem due to Bôcher we prove

THEOREM 6. Under the conditions of Theorem 5, let C and D lie, respectively, in disjoint circular regions S and T . Then all critical points of $u(x, y)$ in R lie in S and T . If C and D consist, respectively, of m and n

components, then S and T contain, respectively, $m - 1$ and $n - 1$ critical points.

At a point z exterior to S and T the force due to the positive mass on C is equal to the force at z due to the same mass concentrated at some point Q_1 of S , by the Lemma, and the force at z due to the negative mass on D is equal to the force at z due to the same mass (numerically equal to the total positive mass) concentrated at a point Q_2 of T ; the field due to these two masses at Q_1 and Q_2 has as lines of force the circles through Q_1 and Q_2 , so the total force at z is not zero. Study of the variation in the direction of the force as z traces a circle separating S and T , and traces loci $u(x, y) = \epsilon$ and $1 - \epsilon$ in R , where ϵ is small but positive, completes the proof.

4. In Theorems 5 and 6 the constant values zero and unity on C and D may be replaced by any other distinct constant values; such a change may modify $u(x, y)$ by an additive constant, which alters u_0 in (3) but leaves (4) unchanged, and may modify $u(x, y)$ by a multiplicative constant, which multiplies u_0 , $v(x, y)$, and hence $d\sigma$ by this same constant, and leaves (3) and (4) formally unchanged; even if the multiplicative constant is negative the conclusion of Theorem 5 persists. This remark will be useful in the proof of

THEOREM 7. *Let $u(x, y)$ be harmonic in a region R bounded by a Jordan curve J and a Jordan configuration B disjoint from J , continuous on $R + J + B$, zero on J and unity on B . Let the region R_1 bounded by J and containing R be provided with non-euclidean (NE) geometry by mapping R_1 conformally onto the interior of a circle, and let Π be the smallest NE convex set in R_1 containing B . Then all critical points of $u(x, y)$ in R lie in Π .*

We make the permissible assumption that J is the unit circle and that the origin is a point of R . We extend $u(x, y)$ harmonically across J so that $u(x, y)$ is harmonic in the region R' bounded by B and the reflection B' of B in J ; then $u(x, y)$ is continuous on $R' + B + B'$, equal to unity on B and minus unity on B' . Both B and B' are finite. The values of $d\sigma$ are equal in corresponding points of B and B' , so the integral corresponding to (4) for R' can be taken merely over B , with the integrand

$$\frac{1}{z - t} - \frac{1}{z - 1/\bar{t}}. \quad (5)$$

The conjugate of (5) is the force at z due to unit positive and negative masses at t and $1/\bar{t}$, and this force has the direction of the circle through z , t and $1/\bar{t}$, in the sense from t toward z . If z is a point of R exterior to Π , there exists a NE line L through z disjoint from Π , and each vector represented by the conjugate of (5) with t on B has a non-zero component orthogonal to L directed toward the side of L on which Π does not lie. Thus z is not a position of equilibrium and Theorem 7 is established. It

follows also that no boundary point of Π is a critical point unless Π reduces to a segment of a NE line.

If B in Theorem 7 is symmetric in a NE line L , there exist precise analogs of Theorems 3 and 4, where now the Jensen circles are to be taken as NE circles with NE centers on L . Still another related result is

THEOREM 8. *Let $u(x, y)$ be harmonic in a region R bounded by a Jordan curve J and two Jordan configurations B_1 and B_2 disjoint mutually and from J , continuous on $R + J + B_1 + B_2$, zero on J , unity on B_1 , minus unity on B_2 . Let the region R_1 bounded by J and containing R be provided with a NE geometry. If there exists a NE line L separating B_1 and B_2 in R , then the totality of such lines L separates a NE convex set Π_1 containing B_1 from a NE convex set Π_2 containing B_2 ; all critical points of $u(x, y)$ in R lie in Π_1 and Π_2 .*

5. For a region R we denote generically by $G(z, z_0)$ Green's function with pole in z_0 , and we study

$$u(z) = \sum_{k=1}^m \lambda_k G(z, \alpha_k), \quad \lambda_k > 0. \quad (6)$$

THEOREM 9. *Let R be a Jordan region, and let the points $\alpha_1, \alpha_2, \dots, \alpha_m$ of R lie in a smallest NE convex set Π . Then all critical points of $u(z)$ defined by (6) also lie in Π .*

Theorem 9 can be proved by remarking that for large positive M the level locus $u(z) = M$ in R consists of m curves, one about each point α_k ; Theorem 7 then applies; as M becomes infinite the smallest NE convex set containing the locus approaches Π .

In a similar manner, by considering the level loci $u(z) = M$ and $u(z) = -M$, there may be proved

THEOREM 10. *Let R be a Jordan region, and let $\alpha_1, \dots, \alpha_m, \beta_1, \dots, \beta_n$ be points of R which lie in two NE convex sets Π_1 and Π_2 separated by every NE line separating the α_j from the β_k . Then all critical points of*

$$u(z) = \sum_{k=1}^m \lambda_k G(z, \alpha_k) - \sum_{k=1}^n \mu_k G(z, \beta_k), \quad \lambda_k > 0, \mu_k > 0, \quad (7)$$

in R lie in Π_1 and Π_2 .

The situation of Theorem 9 can be studied directly even under a more general hypothesis. In Theorem 1 let z_0 be a finite point of R ; choose $u(x, y)$ in (3) as $G(z, z_0)$, with C as B and D as the locus $G(z, z_0) = M$. When M varies, $v(x, y)$ does not change, and we have $\int_D d\sigma = 1$, so as M becomes infinite we obtain

$$G(z, z_0) = \int_D \log r \, d\sigma - \log |z - z_0| + u_0, \quad \int d\sigma = 1, \\ F(z) = G(z, z_0) + iH(z, z_0) = \int \log (z - t) d\sigma - \log (z - z_0) + u_0,$$

$$F'(z) = \int_B \frac{d\sigma}{z-t} - \frac{1}{z-z_0}, \quad \int_B d\sigma = 1.$$

THEOREM 11. Let R be a region bounded by a finite Jordan configuration B , and let $\alpha_1, \dots, \alpha_m$ be finite points of R . Then the critical points of $u(z)$ defined by (6) are the positions of equilibrium in a field of force due to a spread σ of positive matter of total mass $\Sigma \lambda_k$ on B , and to negative particles of respective masses $-\lambda_k$ at the α_k .

One of many consequences of Theorem 11 is

THEOREM 12. Under the conditions of Theorem 11 let B lie in a circular region S and the α_k in a circular region T disjoint from S . Then all critical points of $u(z)$ in R lie in S and T .

6. As is well known, equation (1) expresses $u(x, y)$ as the sum of a simple potential and a double layer potential; in any case we may write from (1) at least formally²

$$\begin{aligned} u(x, y) &= \frac{-1}{2\pi} \int_B \log r \, dv + \frac{1}{2\pi} \int_B u \, d[\arg(z-t)] \\ &= \frac{-1}{2\pi} \int_B \log r \, dv + \frac{1}{2\pi} [u \arg(z-t)]_B - \frac{1}{2\pi} \int_B \arg(z-t) du, \\ f(z) &= u + iv = \frac{-1}{2\pi} \int_B \log(z-t) dv - \frac{i}{2\pi} [u \log(z-t)]_B + \\ &\quad \frac{i}{2\pi} \int_B \log(z-t) du, \\ f'(z) &= \int_B \frac{d\sigma}{z-t} - \frac{i}{2\pi} \left[\frac{u}{z-t} \right]_B + \frac{i}{2\pi} \int_B \frac{du}{z-t}. \end{aligned} \quad (8)$$

The gradient of $u(x, y)$ is the conjugate of $f'(z)$, for which there is an obvious interpretation as a field of force due to spreads σ and iu of matter over B and particles at the end-points of B . Theorems 1, 5 and 11 reduce this general configuration to simpler manageable situations for suitable $u(x, y)$, and those theorems by no means exhaust the possibilities. For instance, if $u(x, y)$ has the constant value unity on an arc $A: (\alpha, \beta)$ of B , the corresponding last two terms of (8) reduce to

$$-\frac{i}{2\pi} \left[\frac{1}{z-\beta} - \frac{1}{z-\alpha} \right]; \quad (9)$$

the conjugate of (9) is a field of force due to *skew particles* at β and α , of masses $+1$ and -1 , respectively; these particles exert forces at z equal to the inverse distance, in the directions $\arg(z-\beta) + \pi/2$ and $\arg(z-\alpha) - \pi/2$. The lines of force in this field are the circles of the coaxial family determined by α and β as null circles. Such fields of force may be

combined with similar ones, or with such fields as occur in Theorems 1, 5 and 11.

Moreover, if $u(x, y)$ is harmonic interior to a circle C and is extended harmonically across an arc of C on which $u(x, y)$ vanishes, $\partial u / \partial \nu$ has equal and opposite values on the two sides of the complementary arc of C , so over the latter arc the first part of the integral in (1) or (8) vanishes. Several results are consequences of these remarks:

THEOREM 13. *Let the points $\alpha_1, \beta_1, \alpha_2, \beta_2, \dots, \alpha_n = \alpha_0, \beta_n = \beta_0$ lie on the unit circle $C: z = e^{i\theta}$ in the counterclockwise order indicated, and let the function $u(z)$ harmonic and bounded interior to C take a positive constant value γ_k on each arc $\alpha_k < \theta < \beta_k$ and the value zero on each arc $\beta_k < \theta < \alpha_{k+1}$. Let NE lines A_0, A_1, \dots, A_{n-1} be so chosen that A_k is an arc of a circle belonging both to the coaxial family determined by α_k and β_k and to that determined by α_{k+1} and β_{k+1} . Then the NE convex set interior to C bounded by the A_k contains all critical points of $u(z)$.*

These arcs A_k are special cases of W-curves, which are of importance in the entire study of critical points, namely, the locus of points at which the forces due to two groups of particles have the same direction but opposite senses; the groups of particles (here the n groups α_k, β_k) have relations of equality of mass between the elements of a group, but between groups all possible relative masses of constant sign are considered. As in Theorem 13, the W-curves form the boundaries of regions necessarily containing the critical points. A double layer distribution on an arbitrary curve C is the limit of a double layer distribution piecewise constant on C as in Theorem 13; the lines of force for the original distribution are the circles normal to C , and the W-curves are the circles normal to C in two points plus the locus of points z_0 from which two circles tangent at z_0 and normal to C can be drawn.

THEOREM 14. *Let R be a region bounded by the unit circle C and by a Jordan configuration B interior to C . Let the function $u(x, y)$ be harmonic and bounded in R , continuous on $R + B + C$ except in the end-points of an arc A of C , unity on B and the interior points of A , zero on the interior points of $C - A$. Let Π be a NE convex set containing B , and let the set Π_1 consist of Π plus all points of arcs joining A and Π_1 of circles of the coaxial family determined by the end-points of A as null circles. Then all critical points of $u(x, y)$ in R lie in Π_1 .*

The conclusion of Theorem 14 applies also to an arbitrary linear combination with positive coefficients of the two harmonic measures $\omega(z, A, |z| < 1)$ and $\omega(z, B, R)$.

Theorem 14 is a sharpened analog of Theorem 7; a similar sharpened analog of Theorem 8 also exists, and as in Theorem 13 we may replace a single arc of C by a sum of arcs of C ; both positive and negative values of $u(x, y)$ on B and C may be admitted. Indeed, all the results formulated

in the present note are intended only as illustrations of an extensive theory which the writer plans to develop elsewhere.

¹ For detailed references to the literature, the reader may consult a forthcoming book: Marden, M., *Geometry of the Zeros of a Polynomial*, to be published as a volume of Mathematical Surveys by the American Mathematical Society.

² Equation (8) is Cauchy's integral formula for $f'(z)$, involving an unusual term to include discontinuous $u(x, y)$.

SOME PROPERTIES OF ROTATIONAL FLOW OF A PERFECT GAS

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1. *Introduction.*—Complete analytic solutions of definite boundary value problems of compressible rotational flow are extremely difficult to obtain. Therefore, in the present stage of study of rotational gas flow the inverse, or semi-inverse, approach promises to prove fruitful. We propose to deduce and to study flow patterns satisfying the differential equations of gas flow and having throughout the field certain prescribed geometric, kinematic or physical properties; but will not make them obey any prescribed boundary conditions.

Usually entire systems of flow patterns, rather than individual solutions, result from this type of approach. If an imposed condition is proved not to be satisfied by any possible flow pattern, then this negative result is of significance, as it establishes a general property of all flows satisfying the equations of the problem.

In various fields of fluid mechanics the semi-inverse approach has been used with considerable success. It is sufficient to recall the contributions of Beltrami, Massotti, Jeffreys, Hamel, Oseen, Kampé de Fériet,¹ Bateman and Tollmien.² None of these investigations deals however with the rotational flow of a perfect gas.

The reason for this is probably that such an investigation—unless restricted to cases of uniform stagnation enthalpy—requires complicated and difficult eliminations if based on the familiar equations of the problem.

In the recent investigations of Munk and Prim^{3, 4} this elimination is accomplished with complete generality as far as steady flow of a perfect gas is concerned. This makes the application of the semi-inverse approach to rotational gas flow practicable. Therefore the results of Munk and Prim are used throughout the present paper. The present paper is

a general preliminary report on our investigations, parts of which will be published in detail elsewhere.

2. *Some Properties of the Plane Flow of a Perfect Gas.*—A natural coordinate system for the analysis of problems of plane fluid motion employs as coordinate curves the streamlines ($\xi = \text{const}$) and their orthogonal trajectories ($\eta = \text{const}$). In this coordinate system the vorticity of the reduced velocity field is expressed by

$$|\text{curl } \mathbf{w}| = -\frac{1}{g_1 g_2} \frac{\partial}{\partial \xi} g_2 w \quad (1)$$

($g_1 d\xi$ and $g_2 d\eta$ are components of the vector element of arc length).

The vorticity equation and the continuity equation⁴ take on the following form:

$$\frac{\partial}{\partial \eta} \left[\frac{w^2}{1 - w^2} \frac{\partial \ln (g_2 w)}{\partial \xi} \right] = 0 \quad (2)$$

$$\frac{\partial}{\partial \eta} [g_1 (1 - w^2)^{\beta} w] = 0. \quad (3)$$

These equations enable us to settle certain general questions concerning plane gas flows.

We ask first under what conditions the velocity is constant along each streamline. Since the ultimate velocity is in every case constant along each streamline, our question is equivalent to asking under what conditions the reduced velocity is constant along each streamline.

Introduction of the requirement $w = w(\xi)$ into equations (2) and (3) yields the geometric conditions:

$$\frac{\partial^2 \ln g_2}{\partial \xi \partial \eta} = 0 \quad \text{or } g_2 = a(\xi)b(\eta)$$

$$\frac{\partial \ln g_1}{\partial \eta} = 0 \quad \text{or } g_1 = c(\xi).$$

An analysis of this geometric limitation leads to the following theorem:

THEOREM 1. *In plane steady flow of a perfect gas, in absence of an external field of mass force, the velocity magnitude can have a constant value along each streamline only if the streamlines are concentric circles or parallel straight lines.*

Obviously, in these cases in which $w = \text{const}$ (and hence also $v = \text{const}$) along each streamline, $\text{curl } \mathbf{w}$ and $\text{curl } \mathbf{\bar{v}}$ also have a constant magnitude along the same lines. The question arises whether the converse theorem is also true, that is, whether the only two dimensional flow patterns possible in a perfect gas such that the streamlines are lines of equal vorticity are those in which they are also lines of equal velocity magnitude.

To settle this question we consider first flow with constant reduced vorticity magnitude along each streamline. Crocco's pressure theorem as reformulated⁵ for a general distribution of stagnation enthalpy requires that the pressure remain constant along each streamline if the reduced vorticity does so; that is, $p = p(\xi)$. On the other hand, according to a familiar relation

$$\frac{p}{p_0(\xi)} = (1 - w^2)^{\beta+1}$$

(p_0 denotes the stagnation pressure, which remains constant along each streamline between shock fronts). Hence $|\text{curl } \bar{w}| = f(\xi)$ implies that w must be also a function of ξ alone.

A very similar argument shows that if the lines of equal velocity magnitude (isovels) and the lines of equal vorticity (isocurls) of the reduced velocity field coincide, then they coincide also with the streamlines.

Starting from the vector identity

$$\text{curl } \bar{v} = a(\xi) \text{curl } \bar{w} + \text{grad } a(\xi) \times \bar{w}$$

we have transformed the above statements relating to the reduced velocity field into analogous statements concerning the actual velocity field and proved the following theorems:

THEOREM 2. *In plane steady flow of a perfect gas, in absence of an external field of mass forces, constant vorticity along each streamline implies constant velocity along the same lines.*

THEOREM 3. *In plane steady flow of a perfect gas, in absence of an external field of mass forces, the coincidence of the isovels and the isocurls implies that they coincide also with the streamlines.*

Our three theorems prove that all flow fields having coincident isovels and streamlines, or coincident isocurls and streamlines, or coincident isovels and isocurls are restricted to flows the streamlines of which are concentric circles or parallel straight lines.

In a paper by R. Prim⁶ the question is investigated whether the streamline pattern can form, with its orthogonal trajectories, an isothermal net, that is a net in which $g_1 = g_2$. The following theorem is established.

THEOREM 4. *In plane steady flow of a perfect gas in absence of an external field of mass forces, the streamlines and their orthogonal trajectories can form an isothermal net only if the streamlines are concentric circles, parallel straight lines or radial straight lines: the last case being possible only if the reduced velocity field is irrotational.*

It will be instructive to compare the geometric restrictions imposed by the above four conditions upon the pattern of gas flow with the corresponding restrictions imposed upon incompressible non-viscous and in-

compressible viscous flow. (For all three types of idealized fluids absence of an external field of mass forces is assumed.)

	NON-VISCOUS INCOMPRESSIBLE FLUID ("IDEAL FLUID")	VISCOUS INCOMPRESSIBLE FLUID	PERFECT GAS
Streamlines coincide with isovels	Streamlines concentric circles or parallel straight lines	Answer is not known	Streamlines concentric circles or parallel straight lines
Isocurls coincide with streamlines	Any rotational plane flow has this property (Helmholtz)	(1) Any flow which has a constant curl throughout the field (2) Streamlines concentric circles or parallel straight lines (Kampé de Fériet)	Streamlines concentric circles or parallel straight lines
Isocurls coincide with isovels	Streamlines concentric circles or parallel straight lines	Answer is not known	Streamlines concentric circles or parallel straight lines
Streamlines and their orthogonal trajectories form isothermal net ($g_1 = g_2$)	(1) Any irrotational flow (2) Rotational flow only if streamlines concentric circles or parallel straight lines	(1) Any irrotational flow (2) Rotational flow only if streamlines logarithmic spirals or their limiting cases (concentric circles, radial straight lines, parallel straight lines (Jeffreys, Hamel)	(1) Irrotational flow: streamlines concentric circles parallel straight lines or radial straight lines (2) Rotational flow: streamlines concentric circles or parallel straight lines

3. *On Flow Fields for Which, in a Cylindrical Isothermal Coördinate System All Three Velocity Components Depend Only on One Coördinate.*—For irrotational, plane gas flow this question was investigated by Tollmien.² We have extended the study to rotational flow, at the same time allowing an additional velocity component parallel to the elements of the cylindric coördinate surfaces. In one respect, however, our investigation is less general than that of Tollmien: we have investigated only systems of such cylindrical coördinate surfaces which in the xy plane form an isothermal net, while Tollmien admitted orthogonal nets of any kind.

The main result of the investigation can be summarized in the theorem:

THEOREM 5. *The only isothermal cylindrical system of coordinates for which the reduced velocity components of any steady non-parallel flow of a perfect gas, in absence of an external field of mass forces, are functions of one of the isothermal coordinates only are formed by logarithmic spirals and their limiting cases. Thus the coördinate systems satisfying our kinematical*

conditions are exactly the same as those found by Tollmien for plane potential flows. With reference to this spiral-cylindric coördinate system the differential equations can be reduced to a pair of ordinary non-linear differential equations of the first order.

4. *Massotti Flow*.—Massotti sought incompressible, non-viscous flow patterns for which $v_z = 0$, and for which at the same time $(\text{curl } \bar{v})_z = 0$.

There is a wide variety of compressible flow patterns the reduced velocity vector of which satisfies the corresponding relations $w_z = 0$ and $(\text{curl } \bar{w})_z = 0$. We satisfy the latter equation by setting

$$w_x = \frac{\partial \phi}{\partial x}; \quad w_y = \frac{\partial \phi}{\partial y}.$$

Hence

$$w^2 = \left(\frac{\partial \phi}{\partial x}\right)^2 + \left(\frac{\partial \phi}{\partial y}\right)^2$$

and the continuity equation takes on the form:

$$\Delta_{x,y} \phi [1 - w^2] = \beta \left[\frac{\partial \phi}{\partial x} \frac{\partial w^2}{\partial x} + \frac{\partial \phi}{\partial y} \frac{\partial w^2}{\partial y} \right].$$

Our vorticity equation, $\text{curl} \left[\frac{\bar{w} \times \text{curl } \bar{w}}{1 - w^2} \right] = 0$, is automatically satisfied as $\bar{w} \times \text{curl } \bar{w}$ is obviously perpendicular to the x, y plane and equal to $1/2(\partial w^2/\partial z)$.

Hence the only condition imposed upon ϕ is the continuity equation which is identical with that of irrotational plane compressible flow. We can immediately see that a wide variety of Massotti flows can be built from families of plane irrotational flows in the following way. Assume $\phi(x, y, c_1, c_2, \dots, c_n)$ is the potential of the reduced field of an n -parameter family of irrotational flows. Replace each of these parameters by an arbitrary function of z ; the ϕ thus obtained can be considered as the potential from which the x and y components of families of reduced velocity fields of the Massotti type can be derived.

For example, we have based upon the simple irrotational Prandtl-Meyer flow a pseudo-plane rotational flow past a developable surface.

5. *Beltrami Flows and Other Investigations*.—Beltrami⁷ was the first to investigate velocity fields which satisfy the condition $\bar{v} \times \text{curl } \bar{v} = 0$. In recent memoranda^{8, 9, 10} the present writers investigate flows of perfect gases which satisfy this relation (Beltrami Flow Proper). They found it fruitful to study also the broader family of flows for which $\bar{w} \times \text{curl } \bar{w} = 0$ (Generalized Beltrami Flow).

Further semi-inverse investigations under way include the study of gas

flow patterns for which all streamlines are straight lines or for which the hodograph space degenerates into a surface or a curve. In addition, the basis of these semi-inverse methods, the equations of Munk and Prim, are being generalized to include certain classes of non-steady flow and of compressible substances with a more general state equation.

¹ See, for example, Berker, A. Ratih, "Sur quelques cas d'integration des équation du mouvement d'un fluide visqueux incompressible," Paris et Lille, 1936.

² See Tollmien, W., "Zum Übergang von Unterschall—in Überschallströmungen," *Z. f. Angewandte Mathematik und Mechanik*, **17**, 117-136 (1937).

³ Munk, M. M., and Prim, R., "On the Multiplicity of Steady Gas Flows Having the Same Streamline Pattern," *Proc. Nat. Acad. Sci.*, **33**, 137-141 (1947).

⁴ Munk, M. M., and Prim, R., "On the Canonical Form of the Equations of Steady Motion of a Perfect Gas," Naval Ordnance Laboratory Memorandum No. 9169 (June, 1947).

⁵ Prim, R., "Extension of Crocco's Theorems to Flows Having Non-Uniform Stagnation Enthalpy," Naval Ordnance Laboratory Memorandum No. 9286 (August, 1947); *Phys. Rev.*, Jan. 15, 1948.

⁶ Prim, R., "On the Existence of Steady Gas Flow in Plane Isothermal Streamline Patterns," Naval Ordnance Laboratory Memorandum No. 9267 (August, 1947); *Bull. Amer. Math. Soc.* (forthcoming).

⁷ Beltrami, E., "Considerazioni idrodinamiche," *Rendiconti Istituto Lombardo*, **22**, 121-130 (1889).

⁸ Neményi, P., and Prim, R., "Some Patterns of Vorticose Flow of a Perfect Gas," Naval Ordnance Laboratory Memorandum No. 9219 (June, 1947).

⁹ Neményi, P., and Prim, R., "Some General Properties of Beltrami Flows of a Perfect Gas," Naval Ordnance Laboratory Memorandum No. 9397 (October, 1947).

¹⁰ Neményi, P., and Prim, R., "Some Interesting Cases of Steady Helicoidal Beltrami Flow of a Perfect Gas," Naval Ordnance Laboratory Memorandum No. 9426 (November, 1947).

REPRODUCTIVE DIAPAUSE IN *DROSOPHILA ROBUSTA*

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Introduction.—Studies of microevolutionary processes in natural populations of *Drosophila* have been hampered by a lack of ecological data. For most species of the genus, the basic facts concerning population size, migration, longevity, specific breeding sites, length of breeding season and nature of overwintering populations are unknown. Workers in this field have not ignored these subjects^{1, 2, 3}, but it is clear that preoccupation with other basic problems has generally held up experiments specifically planned to answer these questions. It is, nevertheless, true that a full understanding of natural selection, genetic drift and other processes, as they are oper-

ative in natural populations of *Drosophila*, will not be attained until the ecology of the various species concerned is better known. The data presented in this paper are based largely on observations which were incidental to a study of the gene arrangements and morphology of *Drosophila robusta*, throughout the warm seasons of the year in a single locality near St. Louis, Missouri.

Although of a preliminary nature, these data show that a striking reproductive arrest, or diapause, sets in during the late summer and early autumn in the natural population, despite the fact that temperatures apparently favorable for the breeding of the flies in nature continue ordinarily until the middle of October. The importance of this fact for the interpretation of population dynamics in this species is obviously great, and further, more detailed investigations of this and similar subjects are planned.

Observations.—The specimens of *Drosophila robusta* on which the following observations were made were collected from paper cups baited with ripe banana mash and set in a particular deciduous woods, supporting considerable undergrowth, near Olivette, St. Louis County, Missouri. In 1946, flies were collected over nine periods of variable length between March 5 and December 10 (see first column of table 1). As the offspring of these

TABLE 1
FERTILITY OF MATURE WILD-CAUGHT FEMALES OF *Drosophila robusta*. 1946 COLLECTION

COLLECTION PERIOD	NO. OF MATURE* FEMALES COLLECTED	FEMALES PRODUCING OFFSPRING WITHIN SIX DAYS	
		NO.	PER CENT
I. Mar. 5–Apr. 6	38	27	71.1
II. Apr. 13–May 15	99	69	69.7
III. May 18–Jun. 1	153	120	78.4
IV. Jun. 17–Jun. 19	203	171	84.2
V. Jul. 3–Jul. 9	108	84	77.8
VI. Jul. 23–Aug. 6	75	64	85.3
VII. Aug. 15–Sept. 3	42	29	69.0
VIII. Sept. 13–Sept. 24	52	2	3.9
IX. Oct. 14–Dec. 10	45	0	0.0

* Wild-caught females which had obviously only recently emerged from the pupal case are not included in this table.

wild flies were to be used for the determination of the frequencies of the various gene arrangements and an F_2 generation from the majority of the wild flies obtained, the following procedure was adopted. Each wild-caught female was placed individually into a separate culture vial soon after capture; after three days it was transferred to a fresh culture vial and at the end of six days both vials were examined for the presence of larvae. If larvae were present, the female was recorded as having been inseminated in nature and the larvae were raised, some being used for the cytological determination of the salivary gland chromosome sequences and the re-

mainder saved as a mass F_1 from the original wild female. If no larvae were observed in six days, the female was mated to a male captured on or about the same date and the F_1 handled in a similar manner. From the middle of June on, a considerable number of females were captured which, because of their light coloration, had obviously only recently emerged from the pupal case. These were separated from the "mature" ones, aged for ten days en masse and then mated individually, to males captured on the same day.

Table 1 shows the total number of females, judged as relatively mature, which were caught during the nine collection periods and the per cent of these which were found to have been inseminated in nature, as indicated by the production of offspring in the laboratory within six days. It will be seen that this percentage remains high from March 5 through August 6, drops in the next period (August 15 through September 3) and falls abruptly to a very low figure for females collected after September 13. It is probable, from the 1947 data given below, that most of the flies failing to produce offspring within six days were virgin, although from the 1946 data alone, the possibility of some sort of slowing-down of egg production by inseminated females cannot be excluded. With a few exceptions, however, all the females collected on or after September 13 produced offspring after being mated to males captured at the same time, although in many cases the production of fertile eggs by these females was delayed for a week or more after being placed with a male.

TABLE 2
ELAPSED TIME BETWEEN EMERGENCE AND THE FIRST APPEARANCE OF F_2 LARVAE FOR
 F_1 FLIES FROM WILD-CAUGHT *Drosophila robusta*. 1946 COLLECTION

COLLECTION PERIOD OF F_1 WILD FLIES	NO. OF F_1 s OBTAINED	NO. OF DAYS ELAPSING FROM EMERGENCE OF F_1 FLIES UNTIL F_2 LARVAE WERE PRODUCED		
		MEAN	MODE	RANGE
IV. Jun. 17-Jun. 19	146	14.5	13	7-31
V. Jul. 3-Jul. 9	150	17.2	14	8-50
VI. Jul. 23-Aug. 6	106	19.5	15	7-51
VII. Aug. 15-Sept. 3	94	25.4	21	10-56
VIII. Sept. 13-Sept. 24	109	33.5	27	11-65
IX. Oct. 14-Dec. 10	31	33.0	34	13-55

F_2 generations were obtained in the following manner from most of the above wild-caught females or pairs of wild flies. F_1 flies of both sexes were maintained in mass culture in vials and changed every three days until F_2 larvae were observed. For each of these F_1 s, the date of the latter event, as well as that of the emergence of F_1 flies was recorded as a matter of routine. These data are thus useful for an analysis of the speed with which the F_1 flies reached a condition of active reproduction as indicated by the production of offspring. Table 2 shows that the F_1 s from wild flies caught from August 15 on attained this condition much more slowly than those

collected in June, July and the first week in August. Correspondence between these data and those for the fertility of the wild-caught parental flies (table 1) is striking, and it is clear that the reproductive delay manifested by the parental flies caught on or after August 15 is reflected in their offspring, despite the fact that the latter were kept under uniform laboratory conditions of temperature (25.5°C.).

The above data appear to be significant in view of the fact that in old laboratory stocks of *Drosophila robusta*, sexual maturity (as indicated by willingness to mate) is attained about four days after emergence for females and about eight days for males. In this species, mating appears to occur several days before the females produce offspring, although further study of this characteristic in laboratory stocks is desirable.

TABLE 3
INSEMINATION OF WILD FEMALE *Drosophila robusta* CAPTURED DURING SEPTEMBER AND OCTOBER, 1947

DATE OF CAPTURE	TOTAL NO. OF FEMALES	NO. OF FEMALES INSEMINATED IN NATURE
1. Insemination determined by observation of hatching eggs:		
Sept. 28-Oct. 2	14	0
Oct. 3-5	30	8
Oct. 7-9	27	1
Oct. 10-12	49	1
Oct. 13-15	28	0
2. Insemination determined by dissection:		
Oct. 14-15	18	1
Oct. 16-18	25	1
Oct. 20-22	21	1
Oct. 23-24	17	0
Oct. 25-27	26	0
Total	256	13
Per cent inseminated in nature		5.1

In 1947, collections were made during two periods from the same locality in the same woods. These periods extended from May 19 through June 7 and from September 28 through October 27, respectively. In order to reduce the labor involved and to expedite analysis of the chromosomes, each wild-caught female in the spring collection was placed with a male immediately upon capture. For this reason, the spring data are of no use in the analysis of fertility in nature. All but very few pairs produced offspring in six days; no F_2 generations were raised in 1947.

The fall (1947) collection was analyzed as follows. Females were placed individually into culture vials shortly after capture. At three-day intervals, each was changed to a fresh vial and the surface of the food closely examined under the binocular microscope for the presence of eggs or larvae. The date of the appearance of eggs was recorded, and if no larvae hatched

from these in the six days following, the female was considered to be virgin and was mated to a wild-caught male. In addition to these, 107 females caught between October 14 and October 27 (when the collecting season was ended by the abrupt onset of cold weather) were dissected immediately on being brought to the laboratory. For each of these, notes were made on the size of the ovary, the character and amount of body fat and whether the female had been inseminated in nature, as indicated by the presence of sperm in the ventral receptacles or spermathecae.

Table 3 shows that approximately 95% of the females caught during this period were virgin, as determined either by watching the fate of eggs laid in the laboratory (upper part of table) or by dissection (lower part of table). Of the 107 females which were dissected, furthermore, none had functional ovaries as indicated by the presence of large filamented ovarian eggs, although in two of them the ovaries were medium in size and contained filamented eggs approximately two-thirds mature size. In almost all the females, the ovaries were very small and rounded (approximately 380 μ in length), similar to those of newly emerged flies. A high percentage of the flies, furthermore, had heavy sheets of body fat, sometimes almost completely filling the abdomen. Such a condition is quite unlike that in a newly emerged fly in which the residual pupal fat, in the form of spherical "nodules" is easily recognized. In 13 of the females, nodular fat was found; this was taken as indicative of relative youth, although, in some of these, fat was also present in small sheets.

Of particular interest were the three dissected flies which had been inseminated in nature. Like most of the virgins, these flies had abundant body fat present in sheets and very small ovaries. The latter, however, were peculiarly elongated and flattened apically, while proximally they presented a slightly swollen appearance. This swollen area contained small ovarian ova of a distinct yellowish-brown color, quite unlike the colorless or whitish ova usually encountered. Ovaries of this unusual type were also found in one of the virgin flies.

Of the 148 females collected between September 28 and October 15, 10 had been inseminated in nature and 138 were virgin. The elapsed time between capture and the laying of the first infertile eggs by the virgins was recorded. This was extremely variable, ranging from 2-25 days, the mean number of days elapsing being 10.3. The 10 inseminated females, furthermore, required a similar mean number of days (9.1) before fertile eggs were laid. One of these females showed an elapsed time of 25 days from the time of capture until the first eggs were laid.

Conclusions.—The 1946 data show clearly that, beginning as early as August, wild females of *Drosophila robusta* captured in the fall reproduce much more slowly than those collected in spring and early summer. A similar delay is manifested in the offspring of these flies. The 1947 data

support this conclusion and suggest that the fall population of females, at least, consists principally of virgin flies with undeveloped ovaries and large quantities of body fat. At least some of the inseminated females have very small, peculiar ovaries which suggest regression from a former more highly developed and possibly functional condition. This relationship between the size of the ovary and the amount of body fat closely resembles the condition in the reproductive diapause which has been shown to occur in other insects in the fall (e.g., "dissociation gono-trophique" in *Anopheles maculipennis*⁴).

A somewhat similar diapause has been described for *Drosophila nitens*.⁵ Laboratory strains of this species, despite the fact that they were kept at 25°C., suspended reproduction from November to March for two consecutive years. Bertani⁶ has shown that this reproductive diapause can be broken by subjecting the adult flies to temperatures from 2-5°C. for 10 days. In *Drosophila robusta*, on the other hand, no interruption or delay of breeding in old laboratory stocks has been observed and a mean of ten days of laboratory conditions appears to be sufficient to break the diapause in wild-caught females.

Biologically, the inference to be drawn is that *Drosophila robusta* overwinters as an adult and that this hibernation period is preceded by a physiological change-over from egg production to the deposition of body fat. The factors, whether genetic or environmental or both, which may be responsible for the initiation of the diapause are unknown and will be the object of further investigation. From the point of view of population dynamics, however, the central fact which emerges from these observations is that the fall population of this species consists largely of non-breeding individuals.

¹ Dobzhansky, Th., and Epling, C., *Carnegie Inst. Wash. Publ. No. 554*, pp. 1-46 (1944).

² Dobzhansky, Th., and Wright, S., *Genetics*, **28**, 304-340 (1943).

³ Spencer, W. P., *Ohio Jour. Sci.*, **41**, 190-200 (1941).

⁴ Swellengrebel, N. H., *Ann. Inst. Pasteur*, **43**, 1370-1389 (1929).

⁵ Buzzati-Traverso, A., *Istituto Lombardo di Science e Lettere, Rendiconti*, **77**, 37-49 (1944).

⁶ Bertani, G., *Nature*, **159**, 300 (1947).

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THE PROTEIN OF THE SALIVARY GLAND SECRETION IN DROSOPHILA

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At the beginning of the prepupal stage, the salivary gland of *Drosophila* becomes enormously inflated. This inflation is due to the appearance of a large amount of material in the lumen of the gland. Prior to the prepupal stage this material is in the cytoplasm of the gland cells but not in the lumen of the gland. It is undoubtedly the secretion released from the cells into the lumen soon after the larval stage terminates. The origin and movements of this material will be described in detail elsewhere.

If an inflated gland is isolated from the prepupa and its tissue is broken in water, the secretion flows out, and unless the water is distinctly acid it dissolves quickly, forming a viscous solution. If, on the other hand, an inflated gland isolated from a prepupa is placed in 95% alcohol, the secretion immediately hardens and becomes opaque while the tissue remains transparent. The secretion can be easily isolated in this condition by removing the tissue with needles under the dissecting microscope. The isolated secretion retains the same solubility in water as before alcohol treatment provided it is not kept too long in alcohol. If an isolated secretion is transferred to absolute alcohol, it immediately becomes brittle and loses its solubility in water except at high pH's. In its native state, i.e., before alcohol treatment, most of this material does not dissolve in common lipid solvents.

By separating the inflated glands from about 800 prepupae of *Drosophila melanogaster* in isotonic saline and isolating the secretions as completely as possible from the tissues in 95% alcohol, about 1600 pieces of secretion were collected. These were transferred to ether as soon as isolated from the tissues and exhaustively extracted, and the residue was thoroughly dried. From the weight of 600 pieces of the residue, the average weight of one piece of secretion was found to be approximately 4 μ g. The ether-soluble

fraction of the secretion was found to be very small in proportion to the residue. Its chemical nature will be described elsewhere and only that of the residue will be reported here.

The residue is insoluble in all common organic solvents and even in dilute or concentrated mineral acids unless heated, but it dissolves readily in weak or strong alkali solutions without heating. A sample of residue was dissolved in dilute NaOH solution and the solution was neutralized with HCl and tested for various color reactions with the following results: positive for ninhydrin and biuret reactions; slightly positive for Molisch reaction; and negative for Millon's, Hopkins-Cole and xanthoproteic reactions. These results suggested that the substance is a protein containing an unusually small amount, if any, of aromatic amino acids. The ultra-violet absorption spectra showed no selective absorption between 210 μ and 310 μ . The absence of selective absorption at about 290 μ agrees with the negative color reactions for aromatic amino acids. The absence of selective absorption at 260 μ indicates absence of nucleic acids in the residue. These tests were repeated with several samples with consistently the same results. The residue is, therefore, not significantly contaminated with the gland tissue, for otherwise the nucleic acid would be detected. In fact, microscopic examinations revealed that the number of cells present in the residue was so extremely small that they can be entirely neglected. It may be added that the dissolved residue is precipitated by phosphotungstic acid but not by trichloroacetic acid.

The nitrogen content of the substance was found by micro-Kjeldahl analysis to be 10.8%. This figure was obtained from one sample of 4.8 mg, and it is unusually low for a protein. When samples of the residue were hydrolyzed with 10% HCl for 24 hours with constant boiling of the acid and when the acid was evaporated on a steam bath, a substantial amount of crystalline inorganic salt was found in the dry residue. Although the proportion of the salt in the samples was not determined, it seems that the unusually low figure for the N content found in the above analysis can well be accounted for by the presence of the inorganic salt, and a large amount of glutamic acid and some glucosamine, both of which have low nitrogen content (see below).

As a preliminary to the two-dimensional chromatogram analysis, three samples of small quantities of the residue were hydrolyzed with 10% HCl for 24 hours with constant boiling of the acid and the hydrolyzates were analyzed on filter paper strips, one with collidine and two with phenol as the solvent. For the principle and technique of the partition chromatogram method of protein analysis, the reader is referred to Consden, Gordon and Martin (1944)¹ and to Dent (1946)². In one of the phenol strips an intensely colored band was present at $R_f = 0.21$. In the control strip in which glutamic acid was used, the R_f of the band was 0.20. In the other phenol strip a strongly colored band was also present at about the same

position. In the collidine strip also an intensely colored band was present at $R_f = 0.12$. In the control strip in which glutamic acid alone was used, the R_f of the band was 0.15. These bands in the experimental and control strips are sufficiently close to each other to indicate that the band in the experimental strip represents glutamic acid. Less intensely colored bands were found at various other positions on all three strips.



FIGURE 1

The result of a two-dimensional chromatogram analysis of the acid hydrolyzate of the protein in the secretion of the salivary gland in *Drosophila* is shown. Each circle represents one amino acid or an oxidation product of an amino acid. The hydrolyzate was put at the position of the cross at the lower right and phenol and collidine were run in the respective directions indicated by the arrows. A, cysteic acid; B, aspartic acid; C, glutamic acid; D, serine; E, glycine; F, threonine; G, alanine; H, tyrosine; I, glucosamine; J, methionine sulfoxide; K, valine; L, lysine; M, arginine; N, proline; O, leucine; P, phenylalanine.

Since most of the bands on the strips represented mixtures of two or more amino acids, a two-dimensional analysis was made to separate all the amino acids contained in the residue in order to identify them individually. Eight hundred μg . of the residue was taken for this purpose. This is about ten times as much as the amount required for an adequate analysis of an

ordinary protein by the chromatogram method. The residue was hydrolyzed with 10% HCl for 24 hours in a sealed glass tube placed in boiling water and the hydrolyzate was analyzed on a square filter paper with phenol and collidine as solvents. The result is shown in figure 1. Sixteen ninhydrin-positive spots are recognized, fifteen of which are identified as amino acids. The color of these spots is, on the original paper, only about half as intense as would be expected if the sample consisted entirely of a protein of usual N content. This indicates the presence in the residue of a considerable amount of ninhydrin-negative substance. The presence of a large quantity of inorganic salt in the residue could account also for this result.

One spot (*I*) is at a position which no known amino acid occupies. This spot corresponds exactly to that of glucosamine. A very weak, positive Molisch reaction indicates the presence of a small quantity of carbohydrate in the residue and supports the chromatogram analysis for the presence of glucosamine.

The central fading of the aspartic acid (*B*), glutamic acid (*C*), glycine (*E*) and arginine (*M*) spots denotes very great strengths of color of these spots. From the areas and color intensities glutamic acid appears to be the largest in proportion and threonine (*F*) next to it. Phenylalanine (*P*) and tyrosine (*H*) are of negligible proportion as indicated by the extreme faintness of the spots. Tryptophane is absent on the paper. These facts agree well with the absence of selective absorption at 290μ and the negative aromatic amino acid color reactions. Methionine sulfoxide (*J*) and cysteic acid (*A*) seem to be artefacts produced by oxidation of the protein and these are probably parts of methionine and cystine, respectively, in the original protein.

The presence of glutamic acid in the largest proportion in this protein is a noteworthy characteristic, but not unusual as a number of proteins are known to contain a disproportionately large quantity of this amino acid. The unique feature, however, is the unusually large proportion of threonine (*F*) and serine (*D*). Dr. Dent informs me that he knows of no other protein so far analyzed by the chromatogram method which shows such an intensely colored and broad spot of threonine in relation to the other spots. Although an accurate quantitative determination is not possible, the fact that this spot is so unusually out of proportion indicates an extraordinarily high content of threonine in the protein of the residue. The serine spot is likewise unusually high in intensity in proportion to the other spots, indicating also an extraordinarily large content of this amino acid in the protein.

As pointed out already, this protein together with the ether-soluble substance is secreted into the lumen of the gland as soon as the animal begins to pupate. The secretion remains in the lumen for a short time and disap-

pears. Where it goes and what it is used for is now being investigated. Although no evidence has yet been obtained, the fact that it disappears from the gland at an early pupal stage suggests a possibility that it either enters into the formation of a part of the pupal body or participates in the chemical processes of histogenesis. One possible function it may have would be to provide through digestion a very large amount of glutamine. Glutamine readily enters into transamination reactions and its presence thus would provide an ample source of a variety of amino acids which must be required for the rapid rate of protein synthesis occurring at this stage of development. Similarly, serine which is present in an unusually large proportion in the secretion protein may, after being freed from the protein, become an abundant source of precursors of other amino acids such as glycine and cystine (Stetten, 1942).³ As to threonine, which is contained also in an extraordinarily large amount in this protein, its possible use in histogenesis cannot be surmised, since only relatively little is known concerning the metabolism of this amino acid.

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¹ Consden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.*, **38**, 224 (1944).

² Dent, C. E., *The Lancet*, Nov. 2, 1946.

³ Stetten, A., *J. Biol. Chem.*, **144**, 501 (1942).

LINKAGE STUDIES OF THE RAT. IX. CATARACT

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Cataract is a dominant character discovered in an albino strain of rats by Smith and Barrantine, and described by them in the *Journal of Heredity*, **34**, 8-10, 1943. A stock of cataractous individuals, 3 females and 2 males, was kindly supplied to us for linkage studies in September, 1943.

No indications have been found of linkage between the gene for cataract and any other gene of the rat. The tests made and their results are summarized in table 1. The progress of the tests has been slow because the cataract is recognizable only in individuals which have unpigmented eyes albinos or pink-eyed yellows. Colored individuals must be subjected to

a breeding test in order to ascertain whether or not they are carriers of the gene for cataract.

The test procedure is as follows. A cross is made between a cataractous individual and an individual carrying the other gene to be tested, as for example, Cataract \times Curly. An F_1 individual will carry Ca and Cu in the repulsion relationship.

Such an F_1 animal, if crossed to normal albinos, will produce four classes of offspring, of which the first and fourth will be crossover combinations, $Ca\ Cu$, Ca , Cu , and 0 (neither Ca nor Cu). If the sum of the crossover classes is significantly less than that of the other two classes, linkage will be indicated. In no cross made has such an indication been found. See table 1.

We conclude that the gene for cataract does not lie in any of the four known linkage systems, nor in chromosomes tagged by the genes, Cu_2 , j , d , A and h . This indicates that it will serve as marker of a tenth chromosome.

TABLE 1

TESTS FOR LINKAGE BETWEEN THE DOMINANT GENE CATARACT (Ca) AND OTHER GENES OF THE RAT. KNOWN LINKAGE SYSTEMS INVOLVED IN THE TESTS ARE INDICATED BY ROMAN NUMERALS. FREQUENCIES OF CROSSOVER CLASSES ARE SHOWN IN ITALIC NUMERALS

GENES	PROGENY CLASSES		TOTAL CROSS- OVERS	TOTAL NON-CROSS- OVERS	GRAND TOTAL	DEV./P.E.
	REPULSION	COUPLING				
I $Ca \times p$	$Cap\ Ca$ 99 109		99	109	208	5.0/4.8
II $Ca \times Cu$	$CaCu\ Ca\ Cu\ 0^*$ 12 20 28 34	$CaCu\ Ca\ Cu\ 0$ 19 21 27 28	94	95	189	0.5/4.6
III $Ca \times h$	$Ca h\ Ca\ h\ 0$ 7 5 2 1		8	7	15	0.5/1.3
IV $Ca \times hr$	$Ca hr\ Ca$ 32 26		32	26	58	3.0/2.5
IV $Ca \times wo$	$Ca wo\ wo$ 6 6		6	6	12	0/1.1
$Ca \times Cu_2$	$CaCu_2\ Ca\ Cu_2\ 0$ 111 135 160 158		269	295	564	13.0/8.0
$Ca \times j$	$Ca j\ Ca\ j\ 0$ 13 5 6 8	$Ca j\ Ca\ j\ 0$ 17 16 17 20	54	48	102	3.0/3.4
$Ca \times d$	$Ca d\ Ca\ d\ 0$ 11 17 6 6		17	23	40	3.0/2.1
$Ca \times A$	$Ca A\ Ca\ A\ 0$ 13 4 11 3	$Ca A\ Ca\ A\ 0$ 14 10 11 12	37	41	78	2.0/2.9
$Ca \times h$	$Ca h\ Ca\ h\ 0$ 1 2 3 6	$Ca h\ Ca\ h\ 0$ 3 3 11 5	21	13	34	4.0/1.9

* Showing neither gene.

THE SUPPRESSION OF CROSSING OVER IN INVERSION
HETEROZYGOTES OF *DROSOPHILA PSEUDOOBSCURA*

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Observations and experiments on natural and artificial populations of *Drosophila pseudoobscura* have shown that within a given population flies of which the third chromosomes differ in gene arrangement (inversion heterozygotes) often possess higher adaptive values than those of which the homologous pair has the same arrangement in both chromosomes (inversion homozygotes).^{1, 2} In many localities the inversion heterozygotes outnumber the homozygotes. The welfare of the populations of these localities, therefore, seems to depend upon the maintenance of a high adaptive level in the inversion heterozygotes relative to the homozygotes. In other localities, however, the populations consist mainly or entirely of inversion homozygotes. In these the adaptive level of the homozygotes must be at least tolerably high.

In theory, two genetic mechanisms could bring about adaptive differences between and among the inversion homozygotes and heterozygotes. First, the action of genes in development may be altered simply by differences in their order within the chromosome (position effect). Second, chromosomes with different gene arrangements may be essentially alike but may carry different complexes of genes which make their possessors physiologically and adaptively different. At the same time they might be co-adapted in such a way as to produce the higher adaptive level of the inversion heterozygotes. Of these two mechanisms the first, position effect, may or may not play a rôle in the evolution of the third chromosome inversions of this fly. However this may be, the second is both effective and important. For if the superior adaptiveness of inversion heterozygotes were due to position effect alone, then each arrangement would be expected to have the same relative superiority regardless of the geographic origin of the chromosomes concerned. This is not the case. The evidence shows that inversion heterozygotes are adaptively superior to the corresponding homozygotes only if the chromosome pair has been drawn from the same or neighboring localities. They are no longer superior when each member of the pair is drawn from distant populations (as for example, from southern California and the central Sierra Nevada). The gene contents of the chromosomes from these populations are not co-adapted to each other in such a way as to produce adaptively superior heterozygotes when combined.³

The biological function of inversions in the natural populations of

Drosophila and their principal rôle in the evolution of these insects might be conceived to be the suppression of crossing over between gene complexes which have reached an adaptive equilibrium. Crossing over would destroy these complexes and would result in gene combinations of different adaptive values, but would be prevented by the binding effect of the inversions. Any inversion which is intrinsically neutral, that is, which produces no position effects, would accordingly spread through a population provided that it would guard from disintegration a gene complex well adapted to the habitat concerned. This would be particularly important where the highest adaptive values are found in heterozygotes. The degree to which such gene complexes are maintained by the binding effect of inversions would accordingly depend upon the degree to which crossing over is suppressed. The present study was designed to secure this information.

TABLE 1
RECOMBINATION OBSERVED IN THE CROSS $\frac{or\ Sc\ pr\ cv}{\text{WILD}} \text{♀} \times \frac{or\ Sc\ pr\ cv}{\text{WILD}} \text{♂}$

		STANDARD	ARROWHEAD	CHIRICAHUA	TREE LINE
0	wild type	2035	4473	3748	2708
	$\left\{ \begin{array}{l} or\ pr\ cv \\ or \end{array} \right.$	769	1251	873	558
1	$\left\{ \begin{array}{l} pr\ cv \\ or\ pr \end{array} \right.$	1781	175	14	3
	$\left\{ \begin{array}{l} pr\ cv \\ or\ pr \end{array} \right.$	738	62	1	2
2	$\left\{ \begin{array}{l} or\ pr \\ cv \end{array} \right.$	357	2
	$\left\{ \begin{array}{l} or\ pr \\ cv \end{array} \right.$	425	7
1,2	$\left\{ \begin{array}{l} or\ cv \\ pr \end{array} \right.$	260
	$\left\{ \begin{array}{l} or\ cv \\ pr \end{array} \right.$	311	4
Total		6676	5974	4636	3271

Three wild strains of *Drosophila pseudoobscura* carrying Standard, Arrowhead and Chiricahua gene arrangements, respectively, and a strain carrying the Tree Line arrangement were employed. The former were originally collected at Piñon Flat, San Jacinto Mountains, California, the latter was collected at Mather, California, in the Sierra Nevada. The inversion heterozygotes of both localities are known to be adaptively superior to the homozygotes. The third chromosomes bearing the gene arrangements designated have been described previously.⁴ Flies of each strain were outcrossed to a strain homozygous for the third chromosome recessives *orange* (*or*), *purple* (*pr*), *crossveinless* (*cv*) and the dominant *Scute* (*Sc*). The *or Sc pr cv* chromosome is known to have the Standard arrangement. F_1 females resulting from this cross, were then testcrossed to *or Sc pr cv* males. The progeny counts of the testcrosses are summarized in table 1. The gene *Sc* was disregarded in the counts because its manifestation in heterozygous condition is variable in different strains.⁵

The observed percentages of recombinations are as follows:

INTERVAL	STANDARD	ARROWHEAD	CHIRICAHUA	TREE LINE
<i>or-pr</i>	46.3	4.0	0.3	0.15
<i>pr-cv</i>	20.3	0.2	0	0

Because the distance in the chromosome between the genes *or* and *pr* is so great that considerable double crossing over must take place in the flies with Standard chromosomes, another experiment was arranged. The four wild type strains were crossed to flies which carried a third chromosome with the recessives *or* and *pr* and the dominants *Blade* (*Bl*) and *Sc*. F_1 females showing *Bl* and *Sc* were selected from the progeny and testcrossed to homozygous *or pr* males. The progeny counts of this testcross are shown in table 2.

TABLE 2
RECOMBINATION OBSERVED IN THE CROSS *or Bl Sc pr* ♀ × *or pr* ♂
WILD

	STANDARD	ARROWHEAD	CHIRICAHUA	TREE LINE
0 {wild type	1933	3555	7483	2218
{ <i>or Bl Sc pr</i>	1147	1954	3046	725
1 { <i>or</i>	313	99	27	7
{ <i>Bl Sc pr</i>	220	54	21	..
2 { <i>or Bl</i>	562	17	1	..
{ <i>Sc pr</i>	408	9	2	..
3 { <i>or Bl Sc</i>	802	6
{ <i>pr</i>	922	11
1,2 { <i>Bl</i>	3
{ <i>or Sc pr</i>	6
2,3 { <i>Bl Sc</i>	81
{ <i>or pr</i>	88	1	2	..
2,3 { <i>Sc</i>	84
{ <i>or Bl pr</i>	105
Total	6764	5706	10582	2950

The following percentages of recombination have been computed from the data in table 2.

INTERVAL	STANDARD	ARROWHEAD	CHIRICAHUA	TREE LINE
<i>or-Bl</i>	10.5	2.7	0.5	0.2
<i>Bl-Sc</i>	17.3	0.5	0.03	0
<i>Sc-pr</i>	32.1	0.3	0.02	0

The standard map distance obtained in our experiments between the loci furthest apart, *or* and *cv*, is 80.2 units, or slightly higher than the figure 68.4 units, obtained by Tan⁶ from somewhat less extensive data. It may also be noted that Tan's experiments were conducted at 25°C., ours at ≈18°C. The location in the cytologically visible chromosome of

the genes used in our experiments can be inferred from the chromosome map published by Tan.⁷ As seen in the salivary gland cells, the third chromosome is a ribbon-like structure which for purposes of description has been subdivided into nineteen more or less equally long sections numbered 63 to 81, inclusively. The gene *or* lies in section 65 at a distance from the centromere of about one-tenth the length of the whole chromosomes. The centromere is in section 63. The gene *cv* lies somewhere in sections 79–81, close to the free end of the chromosome. The map distance of 80 units between *or* and *cv* therefore represents most of the genetic length of the third chromosome. Taking into account the fact that some double crossing-over has remained undetected, and also that between one-tenth and one-fifth of the cytological chromosome contains no known genetic markers, the linkage map of this chromosome is probably less than 100 units long.

The total frequency of recombination found in Standard/Arrowhead heterozygotes is about 4.0 per cent, in Standard/Chiricahua 0.5 per cent and in Standard/Tree Line only 0.2 per cent. Arrowhead differs from Standard by a single inversion which includes sections 70–76 or about one-third the length of the chromosome.⁴ Tan⁷ places the gene *Sc* within, and *pr* outside and distal to the Arrowhead inversion, but his data regarding *pr* are inconclusive. *Bl* and *or* certainly lie between the inversion and the centromere. In Standard/Arrowhead heterozygotes less than one-fiftieth of the recombination normally taking place in the *pr-cv* interval is permitted, and about one-fifteenth of recombination between *or* and *pr*, most of it in the *or-Bl* interval. In other words, an inversion exerts a relatively greater suppressive effect on recombination in the part of the chromosome which lies distal to it (between the inversion and the free end of the chromosome) than it does on recombination between the centromere and the inversion. This agrees with the data of Sturtevant⁸ for *Drosophila melanogaster*.

The Chiricahua and Tree Line arrangements differ from the Standard each by a triple inversion which extends from about the middle of section 68 to section 79, inclusive, or about six-tenths of the length of the chromosome as seen in the salivary gland cells.⁴ Our data show that recombination in Chiricahua/Standard and Tree Line/Standard is almost wholly suppressed in the whole of the third chromosome. The small amount still permitted, less than six-tenths per cent, is concentrated between the genes *or* and *Bl*, a negligible amount being found elsewhere. Particularly noteworthy is the fact that crossing-over is very strongly suppressed between the centromere and the inversions, the result, probably, of interference by the inversions with meiotic pairing.⁹ No recombination whatsoever is detectable in the inverted part of the chromosome. (The three *or pr* flies recorded in table 2 among Standard/Chiricahua and

Standard/Tree Line heterozygotes are most likely the result of contamination; otherwise, they must represent triple crossovers, one between *or* and the inversion and a double to include the locus of *pr*; the individuals concerned were not tested further.)

The amount of crossing over within the inverted parts of the chromosome cannot be determined from our data, since single crossing-over in paracentric inversions does not result in detectable recombination.¹⁰ However, single crossing-over in multiple inversions may result in deficiencies and duplications which produce unviable zygotes. The Standard/Tree Line heterozygotes, for example, have a long paired region which extends from section 68 to 74 (see Fig. 3 in Dobzhansky⁴). Crossing-over in this region would result in chromosomes which would act as lethals in zygotes. Whether such crossing over actually takes place is unknown; in neither case would viable chromosomes carrying gene recombinations normally borne in Standard and in Tree Line (or Chiricahua) appear. The possibility cannot be excluded that in inversion heterozygotes chiasmata may be localized in the immediate vicinity of the centromere, but crossing-over at the centromere would give no recombinations of the genes in Standard, Tree Line and Chiricahua chromosomes.

Summary.—Heterozygosis for inversions found in the third chromosome in natural populations of *Drosophila pseudoobscura* reduces the frequency of recombination of genes located in the chromosome to a small fraction of the normal value. Recombination is strongly prevented not only for genes within the inverted sections, but also for those which lie between the centromere and the inversion, and between the inversion and the free end of the chromosome. Inversion is therefore a powerful means of holding together gene combinations which confer upon their carriers superior adaptive properties.

¹ Dobzhansky, Th., *Evolution*, 1, 1-16 (1947).

² Dobzhansky, Th., and Levene, H. Unpublished data.

³ Unpublished data.

⁴ Dobzhansky, Th., in Dobzhansky and Epling, Carnegie Inst. Washington, Publ. 554, 47-144 (1944).

⁵ Helfer, R. G., *Genetics*, 24, 278-301 (1939).

⁶ Tan, C. C., *Ibid.*, 21, 796-807 (1936).

⁷ Tan, C. C., *Z. Zellforsch. u. mikroskop. Anat.*, 26, 439-461 (1937).

⁸ Sturtevant, A. H., Carnegie Inst. Washington, Publ. 421, 1-27 (1931).

⁹ Dobzhansky, Th., *Am. Nat.*, 65, 214-232 (1931).

¹⁰ Sturtevant, A. H., and Beadle, G. W., *Genetics*, 21, 554-604 (1936).

THE RÔLE OF MUTATION AND OF SELECTION IN THE FREQUENCY OF MUTANTS AMONG MICROORGANISMS GROWN ON IRRADIATED SUBSTRATE

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The mutation rate in microorganisms is increased by irradiation or chemical treatment of the substrate.^{1, 2} The problem of differentiating between the selection of spontaneous mutants and the induction of additional mutants is difficult, especially as the mutation rates are low and the growth rate of bacteria on treated substrate may be slower than normal. If selection is to explain the increase in frequency of mutants found in irradiated broth over that found in normal broth, it must increase the reproductive rate of mutants or inhibit it less than it inhibits the growth of normal cells. The increased number of mutants found in irradiated broth must be explained by induced mutation if selection is neutral or against the mutants.

Experimental.—The procedures for irradiating the medium with ultra-violet light and determining the drug resistant mutants are essentially those employed in our earlier reports.^{1, 2} In addition to the stock strain of *Staphylococcus aureus* (F.D.A. #209) the first experiments reported here involved a penicillin resistant strain and a streptomycin resistant strain which arose as mutants when the stock strain was grown in irradiated broth. Identical inocula from 4-hour log phase cultures and also from 24-hour cultures (older cultures do not give consistent results) of each strain were planted in plain nutrient broth (control) and in irradiated nutrient broth. Immediately after inoculation and again after five hours, samples from each bottle were withdrawn for plating to determine the total count and the number of drug-resistant individuals in each population.

In table 1 are given the number of mutants per million organisms which are resistant to the indicated concentrations of the antibiotics, except for the figures in italics which show the total plate counts. The age of the inocula appeared to have little effect on the behavior of the organisms and the two portions of the table may be regarded as replicate experiments. Since these cultures produce a maximum of about 300 million cells per ml. under the conditions employed, it is evident that all cultures were still in the rapid growth phase at the 5-hour plating. The amount of radiation to which the broth was exposed was such that in no case did the culture growing in the irradiated broth fall behind the replicate growing in the control broth by as much as one division during the 5-hour growth period.

TABLE I
EFFECT OF IRRADIATION OF THE BROTH ON THE GROWTH AND MUTATION RATE OF
NORMAL AND DRUG-RESISTANT *S. aureus*

	24-HR. INOCULUM				4-HR. INOCULUM			
	CONTROL		IRRADIATED		CONTROL		IRRADIATED	
	0	5	0	5	0	5	0	5 HRS.
Normal Strain								
Total count, millions*	2.4	75	1.8	52	0.86	65	1.1	47
Penicillin, 0.07 u./ml.	13	19	17	344	7.0	4.6	9.3	40
Streptomycin, 3.0 u./ml.	65	41	79	310	48	23	46	185
Streptomycin, 6.0 u./ml.	42	12	36	123	10	1	2.8	40
Penicillin Resistant Strain								
Total count, millions*	1.5	60	1.7	41	0.73	80	1.1	54
Penicillin, 0.07 u./ml.	18T	999T	17T	850T	14.5T	1.8T	6T	14T
Streptomycin, 3.0 u./ml.	49	40	40	215	53	10	53	196
Streptomycin, 6.0 u./ml.	19	32	12	98	5.5	6.3	3.5	21
Streptomycin Resistant Strain								
Total count, millions*	0.78	68	0.82	53	1.8	67	1.6	60
Penicillin, 0.07 u./ml.	5.1	6.5	16	70	9.2	3.9	21	85
Streptomycin, 3.0 u./ml.	500T	280T	330T	226T	302T	78T	260T	180T
Streptomycin, 6.0 u./ml.	94T	66T	73T	12.8T	80	30	180	775

* Figures in italics are total count in millions determined by plating on nutrient agar. Other figures are the number of mutants (per million of the total count) which grew on the indicated antibiotic concentration. (T = thousand)

Yet during this period the proportion of mutants in the normal population grown in the irradiated broth increased many-fold.

It is clear that the penicillin resistant mutant resembles the normal strain in its rate of mutation to streptomycin resistance under the several test conditions. Likewise, the normal and streptomycin resistant strains have similar mutation rates to penicillin resistance. These rates, measured by rate of occurrence of organisms which grow at the indicated antibiotic concentrations, vary considerably but give a fairly consistent pattern. There is an increase in the number of resistant cells after growth on irradiated broth. However, when the changes in penicillin resistance in the penicillin resistant strain and of streptomycin resistance in the streptomycin

resistant strain under the several test conditions are compared, there is no consistent relation. In only a few instances did all the cells in the so-called resistant strains actually grow in the concentration of the antibiotic employed. Sometimes more appeared under control conditions, sometimes more from the irradiated broth. This is not in harmony with the idea that irradiated broth either conditions or selects for resistant organisms.

Selection could account for the increase in numbers of mutants in the irradiated broth only if the normal bacteria were slowed down in reproductive rate or killed, while the multiplication of the resistant mutants was speeded up or at least not retarded to the same extent. The number of times that the populations increased during the 5 hours was computed from the data on table 1. These figures for the total population and for those fractions which grew in the presence of 0.07 unit per ml. penicillin or 3 units per ml. streptomycin are recorded in table 2.

TABLE 2
INCREASE IN 5 HOURS OF THE TOTAL CELL COUNT AND OF THE MUTANTS EXPRESSED
AS MULTIPLES OF THOSE PRESENT IN THE INOCULUM

	24-HR. INOCULUM			4-HR. INOCULUM		
	CONTROL	IRRADIATED	RATIO	CONTROL	IRRADIATED	RATIO
Normal Strain						
Total count *	31 (5.0)	28 (4.8)	0.9	76 (6.2)	43 (5.4)	0.6
Penicillin mutants	45 (5.5)	575 (9.2)	13	50 (5.7)	185 (7.6)	3.7
Streptomycin mutants (3.0)	20 (4.2)	110 (6.8)	5.5	36 (5.2)	175 (7.4)	5
Penicillin Resistant Strain						
Total count	40 (5.4)	24 (4.6)	0.6	110 (6.8)	49 (5.6)	0.4
Streptomycin mutants (3.0)	33 (5.1)	128 (7.0)	4	21 (4.4)	181 (7.5)	8.6
Streptomycin Resistant Strain						
Total count	87 (6.5)	65 (6.0)	0.7	37 (5.2)	37 (5.2)	1
Penicillin mutants	112 (6.8)	285 (8.2)	2.5	16 (4.0)	150 (7.2)	9.4

* Figures in parentheses give number of bacterial generations required to produce 5-hour population from inoculum.

Growth of the normal and the streptomycin resistant strains in irradiated broth was only slightly less than in control broth but the penicillin mutant was inhibited to a greater extent. Both the penicillin resistant mutant and the streptomycin resistant mutant were originally isolated from irradiated broth. If selection is to account for the increase in number of organisms resistant to antibiotics in irradiated broth, these mutants should have a positive selection value in irradiated broth. This is not the case. Therefore, selection fails to explain the increase in numbers of organisms resistant to antibiotics in the irradiated broth.

Table 2 shows the differential increase in resistant organisms in irradiated broth during the five-hour growth period. This is expressed both as multiplication of the original cells (normal and resistant) during that

TABLE 3
GROWTH AND MUTATIONS IN A MIXED CULTURE OF NORMAL AND MUTANT CELLS

	HRS.	CONTROL		IRRADIATED	
		MUTANTS PER MILLION	TOTAL IN MILLIONS	MUTANTS PER MILLION	TOTAL IN MILLIONS
Normal Strain					
Penicillin, 0.07 u./ml.	0	20	1.3	16.7	0.7
	2	15.8	38	372	7.8
	5	9.8	102	316	38
Penicillin, 0.20 u./ml.	0	0	1.3	0	0.7
	2	0	38	8.6	7.8
	5	0.4	102	4.7	38
Penicillin Resistant Strain					
Penicillin, 0.07 u./ml.	0	826T	0.58	1000T	0.5
	2	1000T	16.3	992T	1.4
	5	895T	67	1000T	13
Penicillin, 0.20 u./ml.	0	0	0.58	0	0.5
	2	0	16.3	203	1.4
	5	6.0	67	238	13
Mixed Culture (0.9 Normal + 0.1 Resistant)					
Penicillin, 0.07 u./ml.	0	58T	1.7	48T	1.2
	2	41T	40	60T	5.3
	5	42T	96	58T	72

TABLE 4
GROWTH AND MUTATIONS IN A MIXED CULTURE OF NORMAL AND MUTANT CELLS

		CONTROL		IRRADIATED	
	HRS.	MUTANTS PER MILLION	TOTAL IN MILLIONS	MUTANTS PER MILLION	TOTAL IN MILLIONS
Normal Strain					
Streptomycin, 3.0 u./ml.	0	25.3	0.8	29.8	0.8
	3	22.2	35	210	5.3
Streptomycin, 10 u./ml.	0	0	0.8	0	0.8
	3	0.6	35	5.1	5.3
Streptomycin Resistant Strain					
Streptomycin, 3.0 u./ml.	0	925T	0.8	965T	0.6
	3	385T	19.4	429T	3.1
Streptomycin, 10 u./ml.	0	210	0.8	165	0.6
	3	66.8	19.4	935	3.1
Mixed Culture (0.9 Normal + 0.1 Resistant)					
Streptomycin, 3.0 u./ml.	0	106T	1.2	124T	1.0
	3	56T	36.0	104T	7.2

period and as cell divisions necessary to give the net increase in numbers. The increase in number of resistant cells is of a consistently different order of magnitude from the differential increase in number of cells present in

irradiated and control media. Furthermore, the resistant mutants are similar to the normal strain in giving a differential increase in number of mutants resistant to the second antibiotic. These differences are expected if some substance in the irradiated broth causes an increase in the mutation rate. They are not in agreement with an explanation of the increase of resistant organisms based on selection.

Since it may be suggested that population dynamics in mixed cultures sometimes gives an unexpected advantage to one component, artificial mixtures of the normal and mutant organisms were prepared. The inoculum of the mixed cultures, which consisted of 9 times as many organisms from the normal strain as from the mutant strain, was planted in both irradiated and plain broth as were the requisite pure culture controls. At the time of inoculation and again 2 and 5 hours, or 3 hours later, the total count and the numbers of the mutants were determined (tables 3 and 4). It is evident that the mutants enjoyed no selective advantage in the irradiated broth. However, there was a definite and greater increase in the resistant strain of the mutants which grew in the higher concentration of antibiotics.

Another line of evidence is derived from experiments in which organisms were left in contact with the irradiated broth for varying periods of time and then washed and removed to normal broth to eliminate the possible selective action of inhibitory principles derived from the radiations. The procedure which included controls to test the effect of the washing and transferring process is described below and is outlined in the footnote of table 5.

Bottles of sterile unirradiated and irradiated nutrient broth (I A and II A) were inoculated with about 2×10^6 organisms per ml. from a log phase culture of *S. aureus*. After one-half hour a 10-ml. sample was withdrawn aseptically from each inoculated broth and placed in a sterile centrifuge tube; the bottles were incubated at 37°C. The samples were centrifuged and the sedimented organisms were washed with unirradiated broth and recentrifuged. The washed organisms were inoculated into bottles of unirradiated broth (I B and II B) from which samples were immediately withdrawn and plated in appropriate dilutions to determine the total numbers and the incidence of mutants. Further platings were made after 2- and 5-hour incubation at 37°C.

When the cultures in bottles I A and II A had been incubated 1½ hours, another 10-ml. sample was withdrawn from each and the centrifuging and washing process performed as above. The washed organisms from the 1½-hour cultures were inoculated into bottles of unirradiated broth (I C and II C) from which platings were made as before at 0, 2 and 5 hours after inoculation. In a like manner, a third sample was withdrawn from bottles I A and II A after they had been incubated 4 hours, but due to

TABLE 5
EFFECT OF THE TIME OF CONTACT WITH IRRADIATED BROTH ON SUBSEQUENT GROWTH
AND MUTATIONS IN UNIRRADIATED BROTH

BOTTLE	HOURS AFTER INOCULATION								
	0			2			5		
	a	b	c	a	b	c	a	b	c
Experiment 1									
I A	2	6	32	65	88	177
II A	3	4	22	83	7	11
I B	0.6	16	48	6	65	158	83	46	57
II B	0.7	6	25	10	6	13	79	6	9
I C	0.8	69	159	24	50	115	63	103	374
II C	5.8	4	11	32	3	21	84	8	17
I D	0.8	100	186	12	82	262	63	35	145
II D	1.0	7	30	8	7	38	51	6	16
Experiment 2									
I A	2	17	43	55	300	102
II A	2	12	26	96	1	8
I B	0.3	39	48	15	46	31	47	110	58
II B	0.4	12	31	19	8	6	55	2	8
I C	0.6	59	26	14	19	57	42	255	472
II C	2	15	20	33	1	6	87	4	14
I D	1	78	152	23	148	257
II D	2	2	11	26	6	14

a = total count per ml. in millions. *b* = mutants per million on 0.07 unit per ml. penicillin. *c* = mutants per million on 3 units per ml. streptomycin.

I A. Bottle of irradiated broth inoculated with log phase culture of *S. aureus*.

I B. Bottle of unirradiated broth inoculated with washed centrifuged cells which had grown in I A for $\frac{1}{2}$ hour.

I C. As I B but inoculum grown in I A for $1\frac{1}{2}$ hours.

I D. As I B but inoculum grown in I A for 4 hours.

II A, B, C, D. Same as the I series except that II A was unirradiated broth control.

growth of the culture the washed sedimented cells were diluted before inoculating into the bottles of unirradiated broth (I D and II D) so that the inoculum was about 10^6 cells per ml. The total count and the incidence of mutants in bottles I A and II A were determined immediately after inoculation and again after 5 hours' incubation.

The results of replicate experiments are given in table 5.

It should be emphasized that the value of these tests lies in the fact that bacteria were exposed first to irradiated broth and then transferred to normal broth. The table shows that the technique of centrifuging the bacteria did not modify the results appreciably, for the two controls are essentially alike. Further, the same increase in number of mutants present is apparent in bacteria grown in irradiated broth whether or not they were centrifuged and then grown in unirradiated broth. Also this table shows that if a "high" percentage of mutants were present when the bacteria were transferred into normal broth (i.e., after four hours in irradiated broth)

this frequency was retained as the bacteria grew in normal broth. Further, if the bacteria remained in the irradiated broth for only a short period ($1\frac{1}{2}$ hours or sometimes even $\frac{1}{2}$ -hour), the number of mutants present after growth in normal media increased quite markedly. The irradiated media had affected the bacteria so that the mutation rate, measured in their descendants grown in normal broth, was markedly higher after several cell divisions. Demerec³ observed a similar effect from direct irradiation. These results are in agreement with the hypothesis that some material in the media, when utilized in the cell, causes mutations which can show in the descendants of the cell. The increase in number of mutants after transfer into normal broth is not in agreement with an explanation based on selection for resistant organisms by irradiated broth.

TABLE 6
EFFECT OF IRRADIATED BROTH ON THE OCCURRENCE OF BIOCHEMICAL MUTANTS
From mannitol + to -

STRAIN OF <i>S. aureus</i>	CONTROL			IRRADIATED		
	NO. TESTED	MUTANTS	NO. CELLS PER MUTANT	NO. TESTED	MUTANTS	NO. CELLS PER MUTANT
Rosenbach	4,000	0	..	349	1	349
	801	1	801	328	0	..
	3,976	2	1988	1,064	3	355
	3,477	9	386
	6,816	2	3408	10,974	15	731
	465	0	..	4,350	1	4350
Subtotal	16,058	5	3210	30,542	29	1073
Strain A	1,800	0	..	2,160	3	720
Strain B	4,600	1	4600	7,000	5	1400
Strain C	2,100	0	..	4,320	4	1080
Subtotal	8,500	1	8500	13,480	12	1123
TOTAL	24,558	6	4093	44,022	41	1074
From mannitol - to +						
	1,403	0		3,666	18	
	542	0		6,750	1	
	7,448	0		4,000	3	
	3,666	0		
	15,660	0		
TOTAL	28,719	0		14,416	22	655

The third series of experiments concern a different property of the organism, the ability to utilize mannitol. The normal strain of *S. aureus* used can ferment mannitol; it produces mutants which cannot. These mannitol negative organisms can in turn give rise to mannitol positive strains, presumably by reverse mutation. Table 6 shows that both the

direct and reverse mutation rate are increased by treatment with irradiated broth. The several different strains tested acted essentially alike. Grown in normal media, the combined mutation rate from mannitol + to - is one mutation in 4093; on irradiated media one in 1074; or roughly four times as great. No reverse mutation from - to + was discovered in 28,719 tested organisms grown on unirradiated broth, but 22 were found in 14,416 (1 in 655) among organisms grown in irradiated broth. The tables show the fluctuations in frequency of mutants in the several samples as would be expected from a mutation phenomenon. Although it was not proved that these - to + were reverse mutations, this theory is consistent with the situation in higher organisms. Whatever may be the truth of that matter, it is exceedingly difficult to explain how irradiated broth could select both for and against the ability to ferment mannitol.

Summary.—If selection is to explain the increased occurrence of mutants when *S. aureus* is grown in irradiated broth, the following conditions should be met: (1) Quantitative experiments should show that the mutants have a selective advantage over the normal strain under these conditions. This should be especially evident in a mixed culture. (2) When organisms are centrifuged from irradiated broth after a short exposure period and inoculated into unirradiated broth, the increase in the number of mutants should cease. (3) Both forward and reverse mutations should not be differentially increased by growth in irradiated broth. The results of our experiments do not fulfill any of these conditions. The results are in agreement with the hypothesis that mutations are induced by some factor in the irradiated broth.

¹ Stone, W. S., Wyss, O., and Haas, F., these PROCEEDINGS, **33**, 59-106 (1947).

² Wyss, O., Stone, W. S., and Clark, J. B., *J. Bact.*, **54**, 767-772 (1947).

³ Demerec, M., these PROCEEDINGS, **32**, 36-46 (1946).

ON THE AVERAGE VALUE OF ARITHMETIC FUNCTIONS

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The purpose of this note is to introduce a new method of estimating sums of the form

$$S = \sum_{1 \leq n \leq N} f(g(n)), \quad (1)$$

where $f(n)$ is an arithmetic function such as the Euler totient function, $\phi(n)$, or the Dirichlet divisor function, $d(n)$, and $g(n)$ is either a polynomial

in n , a linear term of the form $ap_n + b$, where p_n is the n th prime, or an exponential term of the form $ak^n + b$.

We shall sketch the method in outline and then give the essential particulars for the sum

$$S = \sum_{1 \leq n \leq N} \sigma(P_k(n)), \quad (2)$$

where

$$\sigma(n) = \sum_{d|n} d, \quad (3)$$

and $P_k(n)$ is a polynomial of degree k with integer coefficients. A general investigation of results of this type will be presented subsequently.

The illustrious Indian mathematician, Ramanujan, was the first to appreciate the importance of the function

$$c_q(n) = \sum_{(p, q)=1} \exp(2\pi i pn/q), \quad 1 \leq p \leq q, \quad (4)$$

in the analytic theory of numbers. He gave many interesting expansions involving these functions, of which the following are a sample:

$$\frac{\sigma(n)}{n} = \frac{\pi^2}{6} \sum_{q=1}^{\infty} c_q(n)/q^2 \quad (5)$$

$$d(n) = \sum_{q=1}^{\infty} c_q(n) \log q/q$$

The formulas seem to constitute an important link between the additive and multiplicative properties of numbers. There are several ways of deriving these striking formulas following methods of Hardy, Estermann, or Ramanujan himself. The author has derived another method which was used to state sufficient conditions for the expansion of a function in such a series. These results have been applied to estimation of the generalized Ingham sums of the form,¹

$$\sum_{1 \leq n \leq N} f(n)f(n+k), \quad (6)$$

Returning to (1), we see that if

$$f(n) = \sum_{q=1}^{\infty} a_q c_q(n), \quad (7)$$

then

$$\begin{aligned} S &= \sum_{1 \leq n \leq N} f(g(n)) = \sum_{q=1}^{\infty} a_q \left\{ \sum_{n=1}^N c_q(g(n)) \right\} \\ &= \sum_{q=1}^{\infty} a_q \left\{ \sum_{(p, q)=1} \left\{ \sum_{n=1}^N e^{2\pi i p g(n)/q} \right\} \right\} \end{aligned} \quad (8)$$

Sums of the form

$$E(N, g, q) = \sum_{n=1}^N \exp(2\pi i p g(n)/q) \quad (9)$$

have been the subject of considerable research in connection with Waring's problem. They are also of great intrinsic interest. The following result, due to Hua,² is fundamental.

LEMMA 1. Let $g(n) = \sum_{k=0}^k a_k x^k$, $(a_0, a_1, \dots, a_k) = 1$. Then

$$\left| E(N, g, q) - \frac{N}{q} \sum_{n=1}^q \exp\left(\frac{2\pi i p g(n)}{q}\right) \right| = O(q^{1-1/k+\epsilon}), \quad (10)$$

where the constant implied by O is independent of N , p , and q , depending only on k and ϵ .

The estimate is most useful for $q \leq N$. Hence, the sum for $f(g(n))$ is broken into two parts, $(1, N)$, and $(N+1, \infty)$. The sum from $N+1$ to ∞ is estimated trivially, if $|a_q|$ is small enough as $q \rightarrow \infty$. For the case of $d(n)$ where the series is no longer absolutely convergent, difficulties arise.

We now use the above Lemma to prove

THEOREM. Let $g(n) = \sum_{k=0}^k a_k x^k$, $(a_0, a_1, \dots, a_k) = 1$. Then as $N \rightarrow \infty$

$$\sum_{n=1}^N \sigma(g(n)) \sim \frac{a_k N^{k+1}}{k+1} \sum_{q=1}^{\infty} \frac{1}{q^2} \sum_{(p,q)=1} \left\{ \sum_{n=1}^q \exp(2\pi i p g(n)/q) \right\}. \quad (11)$$

The case where $(a_0, a_1, \dots, a_k) > 1$ may be treated similarly, requiring only slight attention to details.

Proof: We treat $\sum_1 \sigma(g(n))/g(n)$ first and then use partial summation.

$$\begin{aligned} \frac{\sigma(g(n))}{g(n)} &= \frac{\pi^2}{6} \sum_{q=1}^N \frac{c_q(g(n))}{q^2} + \frac{\pi^2}{6} \sum_{N+1}^{\infty} \frac{c_q(g(n))}{q^2} \\ &= P(n) + R(n). \end{aligned} \quad (12)$$

Let us estimate $R(n)$. It is known, elementarily, that

$$c_q(n) = \sum_{\delta|(q,n)} \delta \mu(n/\delta) \quad (13)$$

Hence

$$|c_q(n)| \leq \sum_{\delta|(q,n)} \delta = \sigma((q, n)) \leq (q, n) d((q, n)) \quad (14)$$

since

$$\sigma(n) = \sum_{\delta|n} \delta \leq n \sum_{\delta|n} 1 \leq nd(n). \quad (15)$$

Thus

$$\begin{aligned} |R| &\leq \frac{\pi^2}{6} \sum_{N+1}^{\infty} (q, g(n)) d((q, g(n))/q^2) \\ &\leq \frac{\pi^2}{6} \sum_{\sigma | g(n)} \sigma d(\sigma) \sum_{(q, g(n)) = \sigma} 1/q^2, \quad N+1 \leq q < \infty. \\ &\leq \frac{\pi^2}{\sigma} \sum_{\sigma | g(n)} \frac{d(\sigma)}{\sigma} \left\{ \sum_{[N+1/\sigma]+2 \leq q < \infty} 1/q^2 \right\} \end{aligned} \quad (16)$$

Considering separately the two cases $\sigma > \sqrt{N}$, $\sigma < \sqrt{N}$, we see that $|R|$ is less than

$$\frac{c_2 d^2(g(n))}{\sqrt{N}} = o(1), \quad (17)$$

since $d(n) = O(n^\epsilon)$ for any $\epsilon > 0$. Thus it is clear that

$$\sum_{n=1}^N R(n) = o(N). \quad (18)$$

Using Hua's theorem, Lemma 1, in connection with $\sum_{n=1}^N P(n)$, and then summation by parts, we obtain the result stated in Theorem 1.

To obtain results for primes, we require estimates for trigonometric sums involving primes. For exponential terms, results concerning primitive roots are needed.

¹ Bellman, R., "On Ingham Sums and Ramanujan Expansions," unpublished.

² Hua, L. K., "On an Exponential Sum," *J. Chin. Math. Soc.*, 2, 301-312 (1940).

AN INVERSION AND REPRESENTATION THEORY FOR CONVOLUTION TRANSFORMS WITH TOTALLY POSITIVE KERNELS

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Let b , $\{a_k\}_1^\infty$ be real constants subject to the sole restriction that $\sum_1^\infty (1/a_k)^2 < \infty$. It is easily verified that the formula,

$$G(t) = \frac{1}{2\pi i} \int_{-i\infty}^{i\infty} \frac{e^{st}}{e^{bs} \prod_1^\infty \left(1 - \frac{s}{a_k}\right) e^{s/a_k}} ds, \quad (1)$$

defines $G(t)$ as a real-valued function of t for $(-\infty < t < \infty)$, and that $G(t) \in C^\infty$. The problem of inverting the convolution transform with $G(t)$ as kernel,

$$f(x) = \int_{-\infty}^{\infty} G(x-t)\phi(t)dt, \quad (2)$$

has been considered by D. V. Widder.¹ In a note² in these PROCEEDINGS he announced that if $\phi(t) \in L$ and if x is a point of continuity of $\phi(t)$ then

$$\lim P_n(D)f(x) = \phi(x), \quad (3)$$

where

$$P_n(D) = e^{(b-b_n)D} \prod_{k=1}^n \left(1 - \frac{D}{a_k}\right) e^{D/a_k},$$

the b_n being any real constants such that $\lim_{n \rightarrow \infty} b_n = 0$. Here the symbol D represents the operation of differentiation and $e^{D/a}$ the operation of translation through a distance $1/a$. In preparing the detailed proof of this result the authors discovered it could be much improved. It is the purpose of the present note to state these more general results. We have succeeded in removing all restrictions from $\phi(t)$ except those required to make the integral (2) converge. We have also been able to treat the Stieltjes case,

$$f(x) = \int_{-\infty}^{\infty} G(x-t)e^{ct}d\alpha(t), \quad (4)$$

where c is an arbitrary real constant, which is inverted, as was to be expected, by

$$\lim_{n \rightarrow \infty} \int_{x_1}^{x_2} e^{-cx} P_n(D)f(x)dx = \alpha(x_2) - \alpha(x_1). \quad (5)$$

I. J. Schoenberg³ has studied the functions $G(t)$ in considerable detail, and some few of our results, although obtained by different methods, overlap with his. When this is the case the priority is his. The functions $G(t)$ defined in (1) are examples of what he has called "totally positive" functions. They do not constitute all such functions, but they virtually do so, and we have therefore borrowed his suggestive terminology.

A kernel $G(t)$ belongs to Class I if there are both positive and negative a_k 's; $G(t)$ belongs to Class II if there are only positive a_k 's and if $\sum_1^\infty (1/a_k) = \infty$; $G(t)$ belongs to Class III if there are only positive a_k 's and if $\sum_1^\infty (1/a_k) < \infty$. Either $G(t)$ or $G(-t)$ belongs to one of these classes.

We shall briefly indicate the new properties of $G(t)$ which we have

established. Theorem 1 may be obtained as a special case of more general results due to Schoenberg.

THEOREM 1. If $G(t)$ is defined as in equation (1), then $G(t)$ is a frequency function with mean b and variance $\sum_1^\infty (1/a_k)^2$; moreover, $G(t)$ is bell shaped, i.e., $(d/dt)^n G(t)$ has precisely n changes of sign.

THEOREM 2. If $G_1(t)$ and $G_2(t)$ are defined as in equation (1) with constants b , $\{a_i\}_1^\infty$ and c , $\{d_i\}_1^\infty$ respectively, and if the $\{d_i\}_1^\infty$ are a subset of the $\{a_i\}_1^\infty$, then $G_1(t) - G_2(t)$ has at most two changes of sign.

THEOREM 3. If $G(t)$ is defined by equation (1) and if $G(t) \in$ Class II then for $L = L(t)$ defined as a function of t by the equation,

$$t = \sum_1^\infty \frac{L}{a_k(a_k + L)},$$

we have for all t sufficiently large, $t > T$,

$$-\frac{d}{dt} \log G(t) = L(t + b + o(1)),$$

where $o(1)$ represents a function of t which approaches zero as t becomes positively infinite.

Using these theorems and other results familiar in specific cases from the theory of the Laplace⁴ and Stieltjes⁵ transforms we have been able to prove

THEOREM 4. If $G(t) \in$ Class I, then if the transform (4) converges for any one value of x it converges for all values of x . Moreover, if x_1 and x_2 are points of continuity of $\alpha(t)$, then the inversion formula (5) is valid. If $c \geq \min_{a_k > 0} [a_k]$, then $\alpha(+\infty)$ exists and x_2 may be taken as $+\infty$. If $c \leq \max_{a_k < 0} [a_k]$, then $\alpha(-\infty)$ exists and x_1 may be taken as $-\infty$.

THEOREM 5. If $G(t) \in$ Class II, then if the transform (4) converges for any one value of x , it converges for all larger values of x so that its region of convergence is of the form $\gamma_c < x < \infty$. [The transform may or may not converge at the abscissa of convergence γ_c .] Moreover, if x_1 and x_2 are points of continuity of $\alpha(t)$, then the inversion formula (5) is valid. If $c \geq \min [a_k]$, $k = 1, 2, \dots$, then $\alpha(+\infty)$ exists and x_2 may be taken as $+\infty$.

THEOREM 6. If $G(t) \in$ Class III, if $\alpha(t)$ is of bounded variation in every closed finite subinterval of $T < t < \infty$, and if the transform (4) converges for some one value of $x > T + b + \sum_1^\infty (1/a_k)$, then it converges for all such x .

Moreover, if x_1 and x_2 are points of continuity of $\alpha(t)$, then the inversion formula (5) is valid. If $c \geq \min [a_k]$, $k = 1, 2, \dots$, then $\alpha(+\infty)$ exists and x_2 may be taken as $+\infty$.

Analogous theorems may be stated for the convolution transform (2), which is inverted by the operator (3) for every point of continuity x of ϕ .

We may further improve these results. Let us list some conditions on the a_k 's and b_n 's.

$$A. \quad \sum_{k=1}^{\infty} |a_k|^{-2} = o \left[\sum_{k=1}^{\infty} a_k^{-2} \right]^{1/2},$$

$$B. \quad b_n = o \left[\sum_{k=1}^{\infty} a_k^{-2} \right]^{1/2},$$

$$C. \quad b_n = O \left[\sum_{k=1}^{\infty} a_k^{-2} \right]^{1/2}.$$

If $\alpha(t)$ is supposed to be normalized, $\alpha(t) = 2^{-1}[\alpha(t+) + \alpha(t-)]$, and if conditions *A* and *B* are satisfied, then (5) holds even for points of discontinuity. If condition *C* is satisfied, then (3) holds for all t in the Lebesgue set of $\phi(t)$ and hence almost everywhere.

These results include as special cases, and in a few instances go beyond, the results obtained for the Laplace and Stieltjes transforms, their iterates,^{6,7} the Meijer transform,⁸ etc. They are substantially best possible results.

The authors have further constructed a comprehensive representation theory. Such theories have been constructed for the Laplace and Stieltjes transforms by D. V. Widder,^{4,5} for the iterated Stieltjes transform by R. P. Boas, Jr., and D. V. Widder⁹ and for the third iterate of the Laplace kernel by E. J. Akutowicz.¹⁰ Dr. Boas⁸ has further announced but not published a similar theory associated with the Meijer transform. The present theory includes all these results as special cases. Since the statements of the theorems are rather long, and since they can be readily surmised by anyone familiar with the foregoing particular theories, we shall state only one typical result.

THEOREM 7. *If $G(t) \in$ Class II then the conditions*

$$1. \quad f(x) \in C^{\infty} \quad (\gamma_c < x < \infty),$$

$$2. \quad f(x) = o(e^{\alpha_2 x}) \quad (x \rightarrow \infty),$$

where $\alpha_2 = \text{Min } [a_k], k = 1, 2, \dots,$

$$3. \quad P_n(D)f(x) \geq 0 \quad (\gamma_c - b + b_n - \sum_1^n 1/a_k < x < \infty) \quad n = 1, 2, \dots$$

are necessary and sufficient that

$$f(x) = \int_{-\infty}^{\infty} G(x-t) d\alpha(t),$$

with $\alpha(t)$ non-decreasing and with abscissa of convergence less than or equal to γ_c .

⁴ Widder, D. V., *Duke Math. J.*, **14**, 217-251 (1947).

⁵ Widder, D. V., *Proc. Nat. Acad. Sci.*, **33**, 295-297 (1947).

- ¹ Schoenberg, I. J., *Ibid.*, **33**, 11-17 (1947).
² Widder, D. V., *Trans. Am. Math. Soc.*, **36**, 107-200 (1934).
³ Widder, D. V., *Ibid.*, **43**, 7-60 (1938).
⁴ Pollard, H., *Duke Math. J.*, **14**, 129-142 (1947).
⁵ Pollard, H., *Ibid.*, **14**, 659-675 (1947).
⁶ Boas, R. P., Jr., *Proc. Nat. Acad. Sci.*, **28**, 21-24 (1942).
⁷ Boas, R. P., Jr., and Widder, D. V., *Trans. Am. Math. Soc.*, **45**, 1-72 (1939).
⁸ Akutowicz, E. J., "The Third Iterate of the Laplace Transform," Thesis, Harvard (1947).

THE LAPLACE TRANSFORM FOR LOCALLY COMPACT ABELIAN GROUPS

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Introduction.—As has been shown by A. Weil¹ and others the classical theory of the Fourier transform for functions defined on the real line has a natural generalization to a theory of a transform taking functions defined on a locally compact Abelian group G into functions defined on the character group \hat{G} of G . This theory includes as special cases the theory of n -tuple Fourier series and the theory of n dimensional Fourier transforms. It is based upon the very simple idea of replacing the function $\exp(iy)$ which occurs in the classical one-dimensional Fourier transform by the character function (t, y) whose value for each t in G and each y in \hat{G} is the value at t of the character y ; and thus defining the Fourier transform of a suitably restricted $f(t)$ as $\int f(t)(t, y)dt$ where integration is with respect to the Haar measure in G .

It is the purpose of the present note to describe how this idea may be further exploited so as to generalize the Laplace transform and the Laurent series in an analogous fashion and thus to connect certain aspects of complex variable analysis with the theory of topological groups in the same way that this is done for real variable analysis by the generalized Fourier transform. It is hoped that ultimately such considerations will throw light on the theory of functions of several complex variables.

At the present time we are in a position only to describe the basic notions and state a few preliminary results. We expect to publish later in another journal a paper giving detailed proofs of the theorems stated here as well as those of others which we hope to obtain.

Real Characters, Linear Functionals and One-Parameter Subgroups.—We shall call a continuous complex valued function z , defined on the

locally compact Abelian group G , a generalized character if $z(t_1 t_2) = z(t_1)z(t_2)$ for all t_1 and t_2 in G . It is evident that every generalized character has a unique representation as the product of two generalized characters, one of which takes its values on the positive real axis and the other on the unit circle. The generalized characters of the latter sort are clearly identical with the characters of the Pontrjagin-van Kampen duality theory and will be referred to as ordinary characters. Generalized characters of the former sort will be called real characters. If r is a real character then the function x obtained by setting $x(t) = \log(r(t))$ has the property* that $x(t_1 t_2) = x(t_1) + x(t_2)$ for all t_1, t_2 in G . A continuous real valued function with this property will be called a linear functional. Clearly the above-described mapping sets up a one-to-one correspondence between the real characters and the linear functionals on G . Now let x be any linear functional on G . For each real number u the function taking t in G into the complex number $\exp(iux(t))$ is clearly an ordinary character $y(u)$. Moreover $y(u_1 + u_2) = y(u_1)y(u_2)$ and y is a continuous function of u . Thus $y(u)$ is a continuous homomorphism of the additive group of the real line into \hat{G} . Such a homomorphism is called a one-parameter subgroup of \hat{G} . It may be proved without great difficulty that every one-parameter subgroup of \hat{G} may be obtained from a linear functional in the manner just described and that there is thus obtained a one-to-one correspondence between linear functionals on G and one-parameter subgroups in \hat{G} . We shall find it convenient to use the same symbol to denote a linear functional and the corresponding one-parameter subgroup and to call the compound object so denoted a vector. If x is a vector, t a member of G and u a real number, then $x(t)$ will denote the value at t of the linear functional associated with x and $x[u]$ will denote the member of G assigned to u by the one-parameter subgroup associated with x . We convert the set of vectors into a real vector space by carrying over the obvious vector space operations in the set of linear functionals. We note that $(x_1 + x_2)[u] = x_1[u]x_2[u]$ and $u_1 x[u] = x[u_1 u]$ whenever x, x_1 and x_2 are vectors and u, u_1 and u_2 are real numbers. The natural question concerning the existence of vectors is answered by the

THEOREM. *Let G be a locally compact Abelian group and let G be its character group. Then the following statements about G and \hat{G} are equivalent.*

(a) For each t in G distinct from the identity e there exists a linear functional x with $x(t) \neq 0$.

(b) For each t in G distinct from e there exists a real character r with $r(t) \neq 1$.

(c) The union of the images of all one-parameter subgroups of \hat{G} is dense in \hat{G} .

(d) \hat{G} is connected.

(e) G is the direct product of a discrete torsion free group and the additive group of an n dimensional vector space.

The Laplace Transform.—We define the generalized Laplace transform by replacing the ordinary characters of the Fourier transform theory by the generalized characters described above. The Laplace transform of a suitably restricted function² f defined on G is then $\int f(t)(t, z)dt$ where (t, z) is the value at t of the generalized character z . In view of the remarks of the preceding section we may write this as $\int f(t) \exp(x(t))(t, y)dt$ where y is an ordinary character and x is a vector. The result may be regarded as a function defined in the direct product of the space \bar{G} of all vectors with \hat{G} . If G is taken to be the additive group of integers then \hat{G} is the multiplicative group of complex numbers of modulus one and \bar{G} may be put in to one-to-one correspondence with the positive real numbers in such a way that the positive real number r corresponds to the linear functional which carries n into $n \log(r)$ and hence to the real character which carries n into r^n . Thus the Laplace transform in this case may be regarded as mapping doubly infinite sequences $\{a_n\}$, $n = \pm 1, \pm 2, \dots$, into functions defined in the complex plane with the origin removed. It is readily seen to coincide with the corresponding mapping defined by the Laurent series. If G is taken to be the additive group of real numbers, $\bar{G} \times \hat{G}$ turns out to be isomorphic to the additive group of all complex numbers and the transform considered here reduces to the classical doubly infinite Laplace transform. Taking finite direct products of these groups with themselves we obtain multiple Laplace transforms and Laurent series in several complex variables. It follows from the theorem of the preceding section that there is little if any point in considering groups for which G is not connected. With this in mind we may say that roughly speaking the generalized Laplace transform defined above is the "direct product" of the classical n -fold doubly infinite Laplace transform and a transform which generalizes a multiple Laurent series to the same extent that a general discrete torsion free group generalizes a direct product of finitely many replicas of the additive group of the integers.

There are many questions that may be asked about the nature of the subset of $\bar{G} \times \hat{G}$ in which $\int f(t) \exp(x(t))(t, y)dt$ is defined. The answers to these depend, of course, upon the sense in which the integral is to exist. We shall remark here only that if the integral is required to be absolutely convergent then the region of existence is the direct product of a convex subset of \bar{G} with the whole of \hat{G} . This, of course, implies at once the well-known facts about the regions of absolute convergence of Laurent series and doubly infinite Laplace transforms.

Analyticity of the Laplace Transform.—Partial differentiation of functions defined on a topological group may be defined by means of one-parameter subgroups. If f is a function defined on \hat{G} and x is any member of \bar{G} the

partial derivative f_x of f with respect to x may be defined as the limit as u tends to zero of $(1/u)(f(yx[u]) - f(y))$ provided that this limit exists in some appropriate sense. If f is the Fourier transform of a suitably restricted function k on G then f_x will be the Fourier transform of the function g on G such that $g(t) = x(t)k(t)$ for all t in G .

Since G is a real vector space it is clear that when it is provided with any reasonable topology there will be a natural one-to-one correspondence between its elements and its one-parameter subgroups and, accordingly, such a correspondence between the elements of $\bar{G} \times \bar{G}$ and the one-parameter subgroups of $\bar{G} \times \bar{G}$. Thus if (x_1, x_2) is a member of $\bar{G} \times \bar{G}$ and F a suitably restricted function defined in $\bar{G} \times \bar{G}$ we may speak of the partial derivative $F_{(x_1, x_2)}$ of F with respect to (x_1, x_2) . It is clear that if u is any real number then the partial derivative of F with respect to $u(x_1, x_2) = (ux_1, ux_2)$ is equal to the product of u and the partial derivative of F with respect to (x_1, x_2) . Now $\bar{G} \times \bar{G}$ may be converted into a complex vector space by defining $(u + iv)(x_1, x_2)$ to be the element $(ux_1 - vx_2, vx_1 + ux_2)$ but it will, of course, not always be true that the above homogeneity relationship will continue to hold for complex multipliers. Given any class of differentiable functions those members F for which $F_{(x_1, x_2)}$ is a complex homogeneous³ function of (x_1, x_2) will be referred to as the analytic functions in that class.

As is to be expected there are theorems connecting the properties of being analytic and of being a Laplace transform. We shall close this section by describing such a theorem. Let f be any function in L^2 on G . Then, as may be proved without difficulty, the set of all x in \bar{G} for which $f(t) \exp(x(t))$ is in L^2 on G is a convex subset K_f of \bar{G} and contains the zero element. We shall call a convex subset of \bar{G} large if it contains the zero element and the smallest linear subspace of \bar{G} containing it is the whole of \bar{G} . We shall call a point x in a large convex subset of \bar{G} an interior point if, for some real number u greater than one, the point ux is also in the convex subset. A function f for which K_f is large will be said to be strongly in L^2 . If f is strongly in L^2 then the function on $K_f \times \bar{G}$ which for each x in K_f is defined for almost all y in \bar{G} as the Fourier transform of $f(t) \exp(x(t))$ we shall call the L^2 Laplace transform of f . Now let K be any large convex subset of \bar{G} and let F be a function defined on $K \times \bar{G}$ so as to be in L^2 on \bar{G} for each x in K . Then for each x in the interior of K the expression $(1/u)(F(x + ux_1, yx_2[u]) - F(x, y))$ may for each (x_1, x_2) be regarded as a one-parameter family of elements of L^2 on \bar{G} defined for u sufficiently small. If this family tends to a limit as u approaches zero for all x interior to K and all (x_1, x_2) in $\bar{G} \times \bar{G}$ and if this limit $F_{(x_1, x_2)}$ is complex homogeneous as a function of (x_1, x_2) we shall say that F is L^2 analytic interior to $K \times \bar{G}$. These definitions having been made, our theorem may be stated as follows.

THEOREM. Let F be defined on $K \times \hat{G}$ where K is a large convex subset of \bar{G} . Then F is L^2 analytic interior to $K \times \hat{G}$ if and only if there exists a function f strongly in L^2 on G such that K_1 contains the interior of K and F coincides⁴ interior to $K \times \hat{G}$ with the L^2 Laplace transform of f . If f does exist it is unique (modulo functions zero almost everywhere) and $F_{(x_1, x_2)}$ is also analytic and is the L^2 Laplace transform of the function g such that $g(t) = f(t)(x_1(t) + ix_2(t))$.

Complex Integration and the Cauchy Formula.—If F is the Laplace transform of a function f on G then for each x in \bar{G} it is the Fourier transform of $f(t) \exp(x(t))$. Thus (formally) $\int (F(x, y)/(t, y)) dy = f(t) \exp(x(t))$ so that $\int (F(x, y)/(\exp(x(t))(t, y))) dy = f(t)$. In other words, for suitably restricted functions, f may be recovered from F by integrating $F(x, y)/(\exp(x(t))(t, y))$ over any subset of $\bar{G} \times \hat{G}$ which lies in the region of definition of F and is of the form $x_0 \times \hat{G}$; the measure being the Haar measure in \hat{G} transferred by the one-to-one mapping $y \rightarrow x_0, y$. It follows that F is determined by its values on any subset of the form $x_0 \times \hat{G}$ and we are led to expect a formula giving $F(x, y)$ in terms of $F(x_0, y)$. If we follow through the formal work of finding f from $F(x_0, y)$ and $F(x, y)$ from f and replacing the Fourier transform of a product by the convolution of the Fourier transforms we get the formula $F(x, y) = \int F(x_0, y_1) K(x - x_0, yy_1^{-1}) dy_1$ where $K(x, y)$ is the Fourier transform of $\exp(x(t))$. Unfortunately, this formalism cannot be made to lead to a theorem since the Fourier transform of $\exp(x(t))$ does not exist. Suppose, however, that ϕ is the characteristic function of a set so small that $\phi(t) \exp(x(t))$ does have a Fourier transform for all x in a suitably large subset of \bar{G} . Then if f vanishes outside of this set so that $f = \phi f$ the procedure described above leads to the formula $F(x, y) = \int F(x_0, y_1) K_\phi(x - x_0, yy_1^{-1}) dy_1$ where $K_\phi(x, y)$ is the Fourier transform of $\phi(t) \exp(x(t))$. This formula may be proved rigorously for suitably restricted functions F . In the case in which G is the additive group of the integers and ϕ is the characteristic function of the non-negative integers it reduces to the classical Cauchy integral formula for the special case in which the closed curve is a circle with center at the origin.

If \bar{G} is finite dimensional (and probably with even a weaker restriction) it is possible to extend the above considerations to the case in which the integration is performed over a much more general subset of $\bar{G} \times \hat{G}$ than one of the form $x_0 \times \hat{G}$. Let ψ be a suitably differentiable function defined throughout \hat{G} and having values in \bar{G} . Let C_ψ be the set of all points of $\bar{G} \times \hat{G}$ of the form $\psi(y), y$ for $y \in \hat{G}$. Then a complex valued measure μ_ψ may be introduced into C_ψ which is of such a nature that theorems can be proved establishing the formulae:

$$f(t) = \int_{C_\psi} (F(x, y)/(\exp(x(t))(t, y))) d\mu_\psi$$

and

$$F(x, y) = \int_{C_\psi} F(x_1, y_1) K(x - x_1, yy_1^{-1}) d\mu_\psi$$

where, in the second integration, x and y are regarded as fixed. As the work necessary to remove obviously too restrictive conditions from the statements of such theorems has not yet been done we shall confine ourselves here to a description of the measure μ_ψ . Let θ be the function taking y in \hat{G} into $\psi(y)$, y in $\bar{G} \times \hat{G}$. Then θ is a one-to-one map of \hat{G} onto C_ψ and hence μ_ψ is completely determined by the measure in G defined by the equation $\tilde{\mu}_\psi(E) = \mu_\psi(\theta(E))$. When ψ is sufficiently differentiable $\tilde{\mu}_\psi$ will be absolutely continuous with respect to the Haar measure in G and hence will be completely described by its Radon Nikodym derivative with respect to this Haar measure. This derivative may be computed from ψ as follows. For each x in \bar{G} the partial derivative ψ_x of ψ with respect to x may be defined in a manner strictly analogous to that used for complex valued functions. The result will be linear in x and will be for each y in \hat{G} a member of \bar{G} . Thus for each y in \hat{G} , $\psi_x(y)$ defines a linear transformation $T_\psi(y)$ of the vector space \bar{G} into itself. It may be extended to a complex linear transformation of $\bar{G} \times \bar{G}$ into itself in a unique and obvious fashion. The sought for Radon Nikodym derivative r is defined by the equation: $r(y) = (1/i^n)$ determinant $(iI + T_\psi(y))$. Here I represents the identity transformation and n is the dimension of \bar{G} . Presumably a measure with similar properties may be introduced into C_ψ when ψ is only assumed to have some property analogous to being of bounded variation. How this is to be done is a matter which we expect to investigate.

Concluding Remarks.—Among other results in our possession at the moment is a generalization to functions on $\bar{G} \times \hat{G}$ of Hadamard's three circles theorem. Its proof may be carried out using the same device used by Zygmund and Tamarkin⁶ in proving Thorin's generalization of the M. Riesz convexity theorem. This is not surprising once one has observed that Thorin's theorem is essentially the Hadamard three circles theorem for functions of n complex variables. We have also investigated the extension to groups of the Hilbert transform and have used this extension to obtain formulae giving $F(x, y)$ in terms of its values on a set of the form $x_0 \times \hat{G}$ which are different from those discussed in the preceding section. These and other matters we intend to discuss in full in the more detailed paper promised in the introduction.

¹ Weil, A., "L'intégration dans les groupes topologiques et ses applications," *Actualités Scientifiques et Industrielles*, No. 809, Paris, 1940.

² Functions mentioned in this note are complex valued except when the contrary is explicitly assumed.

³ This particular way of formulating analyticity was suggested to the author by the treatment of complex manifolds in an unpublished manuscript of C. Chevalley.

⁴ Coincidence here means, of course, having the same value for almost all y for each x .

⁵ Cf. Bochner, S., "Group Invariance of Cauchy's Formula in Several Variables," *Ann. Math.*, **45**, 686 (1944).

⁶ Tamarkin, J. D., and Zygmund A., "Proof of a Theorem of Thorin, *Bull. Am. Math. Soc.*, **50**, 279 (1944).

ON THE ASYMPTOTIC DISTRIBUTION OF THE SUM OF A RANDOM NUMBER OF RANDOM VARIABLES

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We shall state without proof some results on the asymptotic distribution as $\lambda \rightarrow \infty$ of the sum

$$Y = X_1 + \dots + X_N$$

of a random number of independent random variables, where the X_j have the same fixed distribution function $F(x) = P[X_j \leq x]$ and where N is a non-negative integer-valued random variable independent of the X_j , whose distribution function depends on a parameter λ . We shall use the notation

$$\begin{aligned} a &= E(X_j), & c^2 &= \text{Var}(X_j) (0 < c^2 < \infty), \\ \alpha &= E(N), & \gamma^2 &= \text{Var}(N) (0 \leq \gamma^2 < \infty), & M &= (N - \alpha)/\gamma, \\ \theta(t) &= E(e^{itN}), & \sigma^2 &= \alpha c^2 + \gamma^2 a^2, & \delta &= (\gamma a)/\sigma, \\ Z &= (Y - E(Y))/\sqrt{\text{Var}(Y)} = (Y - \alpha a)/\sigma, & \varphi(t) &= E(e^{itZ}). \end{aligned}$$

THEOREM 1. If as $\lambda \rightarrow \infty$

$$\sigma^2 \rightarrow \infty, \quad \gamma = o(\sigma^2), \quad (1)$$

then for any t , as $\lambda \rightarrow \infty$

$$\varphi(t) = \theta(\delta t) e^{-1/2t^2(1-\delta^2)} + o(1). \quad (2)$$

COROLLARY 1. If (1) holds and if as $\lambda \rightarrow \infty$

$$a^2 \gamma^2 = o(\alpha), \quad (3)$$

then for any t ,

$$\lim_{\lambda \rightarrow \infty} \varphi(t) = e^{-1/2t^2}, \quad (4)$$

so that Y is asymptotically normal.

COROLLARY 2. If (1) holds and if M has a non-normal limiting distribution function $G(x)$, so that

$$\lim_{\lambda \rightarrow \infty} \theta(t) = g(t) \neq e^{-1/2t^2}, \quad (5)$$

and if

$$\lim_{\lambda \rightarrow \infty} \frac{\alpha c^2}{\gamma^2 a^2} = s (0 \leq s < \infty), \quad (6)$$

then

$$\lim_{\lambda \rightarrow \infty} \varphi(t) = g\left(\frac{t}{\sqrt{1+s}}\right) \cdot e^{-1/2t^2(s/1+s)} \quad (7)$$

so that Z has a non-normal limiting distribution function.

It is easy to show that if M has a limiting distribution function $G(x)$ such that $G(x) > 0$ for every x , then as $\lambda \rightarrow \infty$, $\alpha \rightarrow \infty$ and $\gamma = o(\alpha)$, so that (1) holds.

COROLLARY 3. *If N is asymptotically normal then Y is asymptotically normal.*

An example in which (1) does not hold is the following: for any positive integer λ let N have the values $\lambda, 2\lambda$ with $P[N = \lambda] = P[N = 2\lambda] = 1/2$, and let $a = 0$. Then $\sigma^2 = (3\lambda c^2)/2$, $\gamma = \lambda/2$, $\gamma \neq o(\sigma^2)$. Here we may show directly that

$$\lim_{\lambda \rightarrow \infty} \varphi(t) = 1/2 \{e^{-1/2t^2} + e^{-1/2t^2}\},$$

which is the characteristic function of a mixture of two different normal distributions. This is a special case of the following theorem.

THEOREM 2. *If*

$$a = 0, \quad \lim_{\lambda \rightarrow \infty} \gamma/\alpha = r (0 < r < \infty), \quad (8)$$

and if M has a limiting distribution function $G(x)$ (necessarily such that $G(x) = 0$ for some x), then

$$\lim_{\lambda \rightarrow \infty} \varphi(t) = \int_0^\infty e^{-1/2t^2x} dG_1(x), \quad (9)$$

where

$$G_1(x) = G\left(\frac{x-1}{r}\right). \quad (10)$$

It follows that Z has the limiting distribution function

$$H(x) = \int_0^\infty H_0\left(\frac{x}{\sqrt{y}}\right) dG_1(y), \quad (11)$$

where

$$H_0(x) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^x e^{-1/2u^2} du \quad (12)$$

is the normal distribution function with means 0 and variance 1.

A full account of these results will be published elsewhere.

ON VARIATION-DIMINISHING INTEGRAL OPERATORS OF THE CONVOLUTION TYPE

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A real matrix $A = \|a_{ik}\|$, ($i = 1, \dots, n$; $k = 1, \dots, m$), is said to be *totally positive* if all its minors, of any order, are non-negative. The algebraic properties of totally positive matrices have been intensively studied by F. Gantmakher and M. Krein in 1937.¹ In 1930 the author² showed that if A is totally positive, then the linear transformation,

$$y_i = \sum_{k=1}^m a_{ik} x_k \quad (i = 1, \dots, n), \quad (1)$$

is *variation-diminishing* in the sense that if $v(x_k)$ denotes the number of variations of sign in the sequence $\{x_k\}$ and $v(y_i)$ the corresponding number in the sequence $\{y_i\}$, then we always have the inequality

$$v(y_i) \leq v(x_k). \quad (2)$$

In a previous note³ the author has defined the class P of the so-called *Polya frequency functions* as the class of functions $\Lambda(x)$, $-\infty < x < \infty$, satisfying the following three characteristic conditions:

1. $\Lambda(x)$ is measurable.
2. If $x_1 < x_2 < \dots < x_n$ and $t_1 < t_2 < \dots < t_n$, then the determinant of order n , whose element in the i th row and j th column is $\Lambda(x_i - t_j)$, should be non-negative, i.e.,

$$\det \|\Lambda(x_i - t_j)\| \geq 0. \quad (3)$$

This inequality should be verified for $n = 1, 2, 3, \dots$

3. Finally

$$0 < \int_{-\infty}^{\infty} \Lambda(x) dx < +\infty.$$

All Polya frequency functions $\Lambda(x)$ turn out to be everywhere continuous with the single exception of the so-called truncated exponential

$$\Lambda(x) = \begin{cases} e^{-x} & \text{if } x \geq 0 \\ 0 & \text{if } x < 0, \end{cases}$$

and all functions arising from it by a change of scale and origin. As stated in the same note, a function $\Lambda(x)$ is equivalent (or equal almost everywhere)

to a Polya frequency function if and only if it has a bilateral Laplace transform of the form

$$\int_{-\infty}^{\infty} e^{-sx} \Lambda(x) dx = \frac{1}{\Psi(s)}, \quad (-a < \operatorname{Re} s < a, \text{ for some } a > 0), \quad (4)$$

where $\Psi(s)$ is an entire function of the type II of Laguerre, Polya and Schur⁴

$$\Psi(s) = C e^{-\gamma s^2 + \delta s} \prod_{\nu=1}^{\infty} (1 + \delta_{\nu} s) e^{-\delta_{\nu} s}, \quad (4')$$

(where $C > 0$, $\gamma \geq 0$, δ, δ_{ν} real, $0 < \gamma^2 + \sum \delta_{\nu}^2 < \infty$), the relation (4) holding in a certain open vertical strip of the s -plane containing the imaginary axis. Accordingly

$$\Lambda(x) = e^{-x^2}, e^{-|x|}, e^{x-e^x}, 1/\cosh x,$$

are a few examples of Polya frequency functions because their bilateral Laplace transforms are readily found to be of the form (4), (4').

The analogy between the property (3) of a Polya frequency function and the definition of a totally positive matrix A , as given in the first paragraph, would suggest that the convolution transformation,

$$g(x) = \int_{-\infty}^{\infty} \Lambda(x-t) f(t) dt, \quad (5)$$

should be variation-diminishing, provided $\Lambda(x) \in P$. For this purpose we require the following two definitions.

I. Let $f(x)$ be a real function defined for all real x . The number $v(f)$, of variations of sign of $f(x)$ in the range $(-\infty, \infty)$, is defined as follows: If $S: x_1 < x_2 < \dots < x_n$ is an arbitrary finite increasing sequence of real numbers, then

$$v(f) = \sup_S v(f(x_i)), \quad (0 \leq v(f) \leq \infty).$$

II. Let $\Lambda(x)$ be real and measurable for all real x . We say that the transformation (5) is variation-diminishing if and only if $\Lambda(x)$ enjoys the following two properties:

1.

$$0 < \int_{-\infty}^{\infty} |\Lambda(x)| dx < \infty.$$

2. If $f(t)$ is bounded and measurable, then (5) should always imply the inequality

$$v(g) \leq v(f). \quad (6)$$

The following theorem holds.

THEOREM. *The convolution transformation*

$$g(x) = \int_{-\infty}^{\infty} \Lambda(x-t)f(t)dt \quad (5)$$

is variation-diminishing if and only if the function $\Lambda(x)$ admits of a bilateral Laplace transform of the form

$$\int_{-\infty}^{\infty} e^{-sx} \Lambda(x) dx = \frac{\epsilon}{\Psi(s)} \quad (7)$$

where $\epsilon = \pm 1$ and $\Psi(s)$ is an entire function of the Laguerre-Polya-Schur type (4'), the relation (7) being valid in a certain open vertical strip of the s -plane containing the imaginary axis. An equivalent formulation is as follows: (5) is variation-diminishing if and only if $\epsilon \Lambda(x)$ ($\epsilon = +1$ or else $= -1$) is almost everywhere equal to a Polya frequency function.

If (5) is variation-diminishing, we always have $v(g) \leq v(f)$, even if $f(t)$ is not bounded, provided $f(t)$ is summable in every finite range and such that the improper integral (5) converges for every value of x . In particular, this is always the case if $f(t)$ is a polynomial.

The variation-diminishing property of the classical transformation,

$$g(x) = \frac{1}{\sqrt{\pi}} \int_{-\infty}^{\infty} e^{-(x-t)^2} f(t) dt,$$

has previously been established by Polya⁶ in connection with related work of Laguerre.

In the case of the "rectangular" frequency function

$$\Lambda(x) = \begin{cases} 1/(2h) & \text{if } -h \leq x \leq h \\ 0 & \text{if } x > h, \text{ or } x < -h, \end{cases} \quad (8)$$

(5) turns into the familiar "integral-mean" transformation

$$g(x) = \frac{1}{2h} \int_{x-h}^{x+h} f(t) dt \quad (9)$$

which is often used for practical or theoretical smoothing purposes, a fact which may justify the following remarks. The transformation (9) is *not* variation-diminishing, because (8) is not a Polya frequency function. Indeed, the transform of (8) is

$$\frac{1}{2h} \int_{-h}^h e^{-sx} dx = \frac{\sinh(sh)}{sh}$$

which is certainly not of the necessary form (7) because it has (infinitely many) zeros. An example when (9) increases the number $v(f)$ is easily constructed as follows. Let n be an integer, $n \geq 3$, and let

$$f(x) = \begin{cases} 1 + \cos x & \text{if } x < -\pi \text{ or } x > \pi, \\ 1 + \cos x - (n + 1/2) & \text{if } -\pi \leq x \leq \pi. \end{cases}$$

Evidently $v(f) = 2$. However, with $2h = (2n + 1)\pi$, we find that (9) transforms our $f(x)$ into $g(x)$, where

$$g(x) = \frac{(-1)}{h} \cos x \text{ in the range } -(n - \frac{1}{2})\pi \leq x \leq (n - \frac{1}{2})\pi.$$

Thus $v(g) \geq 2(n - \frac{1}{2}) > v(f)$.

The term "Polya frequency functions" for the functions of our class P seems justified for the following reasons. Let

$$\frac{1}{\Psi(s)} = \sum_{\nu=0}^{\infty} \frac{(-1)^{\nu}}{\nu!} \mu_{\nu} s^{\nu}, \quad (\mu_0 \neq 0), \quad (10)$$

be the power series expansion of the meromorphic function (4). Evidently

$$\mu_{\nu} = \int_{-\infty}^{\infty} \Lambda(x) x^{\nu} dx, \quad (\nu = 0, 1, 2, \dots). \quad (11)$$

If $f(t)$, of (5), is a polynomial of degree n , then (5) may be evaluated as follows:

$$g(x) = \int_{-\infty}^{\infty} \Lambda(t) f(x - t) dt = \int_{-\infty}^{\infty} \Lambda(t) \sum_{\nu=0}^n \frac{(-1)^{\nu} t^{\nu}}{\nu!} f^{(\nu)}(x) dt$$

or

$$g(x) = \mu_0 f(x) - \frac{1}{1!} \mu_1 f'(x) + \dots \pm \frac{1}{n!} \mu_n f^{(n)}(x) = \frac{1}{\Psi(D)} f(x).^6 \quad (12)$$

In this connection Polya proved in 1915 the following remarkable result⁷: "Let (10) be a formal power series (*formal* means that no convergence is assumed) with the following property (S): If $f(x)$ is an arbitrary real polynomial, then the number of real zeros of the transformed polynomial $g(x)$, given by (12), should never exceed the number of real zeros of the given polynomial $f(x)$. A power series (10) enjoys the property (S) if and only if it is the Taylor expansion of the reciprocal $\neq 1/\Psi(s)$ of an entire function $\Psi(s)$ of the form (4')." Thus our Theorem was discovered by Polya in 1915 for the case when $f(x)$ and $g(x)$ are polynomials,⁸ the convolution transformation (5) being replaced by the differential operator relation (12). These remarks show that the Polya frequency functions $\Lambda(x)$ appear implicitly in Polya's work of 1915 in terms of their moments (11).

In concluding we wish to mention a result of Th. Motzkin which is used in the proof of our Theorem. In 1930 the author showed that the linear transformation (1) is certainly variation-diminishing if the matrix A does not possess two minors of equal orders and of opposite signs. He also showed that conversely, if (1) is variation-diminishing, then A cannot have two minors of equal orders and of opposite signs, *provided the rank of A is $= m$* . The necessary and sufficient conditions in order that (1) be variation-diminishing were discovered in 1933 by Th. Motzkin.⁹ They

are as follows: Let r be the rank of A ; then A should not have two minors of equal orders and of opposite signs if their common order is $< r$, while if their common order is $= r$ then again they should never be of opposite signs if they belong to the same combination of r columns of A .

In his contribution to the Courant Anniversary Volume¹⁰ the author has recently discussed the discrete analog of the problem of the present note, namely, the variation-diminishing (sequence) convolution transformations

$$y_n = \sum_{\nu=-\infty}^{\infty} L_{n-\nu} x_{\nu}, \quad (n = \dots, -1, 0, 1, \dots), \quad (13)$$

in their relation to the sequences $\{L_n\}$ satisfying the following two conditions: (1) The 4-way infinite matrix $\|L_{n-\nu}\|$ has only non-negative minors; (2) $0 < \sum_{-\infty}^{\infty} L_{\nu} < \infty$. These sequences are the discrete analogs of the Polya frequency functions.

¹ Gantmakher, F., and Krein, M., "Sur les matrices complètement non négatives et oscillatoires," *Compositio Math.*, **4**, 445-476 (1937).

² Schoenberg, I. J., "Über variationsvermindernde lineare Transformationen," *Math. Z.*, **32**, 321-328 (1930).

³ Schoenberg, I. J., "On Totally Positive Functions, Laplace Integrals and Entire Functions of the Laguerre-Polya-Schur Type," *Proc. Nat. Acad. Sci.*, **33**, 11-17 (1947).

⁴ Polya, G., and Schur, I., "Über zwei Arten von Faktorenfolgen in der Theorie der algebraischen Gleichungen," *J. Math.*, **144**, 89-113 (1914).

⁵ Polya, G., "Qualitative über Wärmeausgleich," *Z. angew. Math. u. Mech.*, **13**, 125-128 (1933); and also Polya, G., "Sur un théorème de Laguerre," *Compt. rend.*, **156**, 996-999 (1913).

⁶ In the present case when $f(x)$, and therefore also $g(x)$, is a polynomial, we may invert the relation (12) by means of the relation $f(x) = \Psi(D)g(x)$. Thus we obtain the inversion of the convolution transformation (5) by the relation $f(x) = \Psi(D)g(x)$, provided $\Delta(x)$ is a Polya frequency function. Polya frequency functions play an important rôle also in Widder's recent work; indeed, he shows that if $\Delta(x)$ is a Polya frequency function then (5) is again inverted by $f(x) = \Psi(D)f(x)$, provided $f(x)$ is merely assumed to be continuous and absolutely integrable in $(-\infty, \infty)$ and the relation $f(x) = \Psi(D)f(x)$ is suitably interpreted. See Widder, D. V., "The Inversion of a Generalized Laplace Transform," *Proc. Nat. Acad. Sci.*, **33**, 295-297 (1947).

⁷ Polya, G., "Algebraische Untersuchungen über ganze Funktionen vom Geschlechte Null und Eins," *J. Math.*, **145**, 224-249 (1915), especially page 231.

⁸ Polya's statement in terms of the numbers of *real zeros* of the polynomials $f(x)$ and $g(x)$ is really equivalent to our statement in terms of the numbers of *variations of sign* of these functions, as we may easily show the equivalence of these two statements as far as polynomials are concerned.

⁹ Motzkin, Th., *Beiträge zur Theorie der linearen Ungleichungen*, (Doctoral dissertation, Basel 1933), Jerusalem, 1936, 69 pp., especially Chap. IV.

¹⁰ Schoenberg, I. J., "Some Analytical Aspects of the Problem of Smoothing," *Courant Anniversary Volume*, New York, 1948, 351-370, especially §2. On page 362 (*loc. cit.*) the transformation (13) is said to be variation-diminishing if: (1) $L_n \rightarrow 0$ exponentially

if $n \rightarrow \pm \infty$; (2) for every bounded sequence $\{x_n\}$, (13) implies the inequality $v(y) \leq v(x)$. The first condition is unnecessarily restrictive; indeed, it is readily seen that Theorem 2 (*loc. cit.*, page 363) and its proof remain valid if we define a variation-diminishing transformation (13) by the following two conditions: (1') $0 < \Sigma |L_n| < \infty$, $L_n > 0$ for some n ; (2') $v(y) \leq v(x)$ for bounded $\{x_n\}$.

PHYSICAL CURVES IN GENERALIZED FIELDS OF FORCE*

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1. The differential geometry of the dynamical trajectories and certain important physical systems of curves of general positional fields of force has been developed by Kasner in his Princeton Colloquium Lectures.¹ Recently we have introduced the concept of *generalized* fields of force which depend not only on the position of the point but also upon the direction through the point. Positional fields may be described as *isotropic* and generalized fields as *anisotropic*.²

If (ϕ, ψ) are the rectangular components of a generalized field of force in the plane, the corresponding equations of motion of a particle are

$$\frac{d^2x}{dt^2} = \phi(x, y, p), \quad \frac{d^2y}{dt^2} = \psi(x, y, p). \quad (1)$$

The cartesian coördinates of a point are denoted by (x, y) , the slope through the point by $p = dy/dx$, and the time by t . We assume that the slope of the force vector is *not* identical with p . For otherwise, the trajectories are all straight lines and the field of force is of no interest. In a generalized field of force, there are ∞^3 trajectories, just as in the positional case.

Upon eliminating the time t from the differential equations (1) of motion of a particle, we find that the differential equation of third order defining a generalized dynamical family of ∞^3 curves is

$$(\psi - p\phi) \frac{d^3y}{dx^3} = [\psi_x + p(\psi_y - \phi_x) - p^2\phi_y] \frac{d^2y}{dx^2} + [\psi_p - p\phi_p - 3\phi] \left(\frac{d^2y}{dx^2} \right)^2. \quad (2)$$

2. A system S_k of curves in a generalized field of force consists of curves along which a constrained motion is possible so that the pressure P is proportional to the normal component N of the force vector. Thus $P = kN$. There are ∞^3 curves in a given system S_k for a fixed k .

The differential equation of third order defining any system S_k is

$$(\psi - p\phi) \frac{d^3y}{dx^3} = [\psi_x + p(\psi_y - \phi_x) - p^2\phi_y] \frac{d^2y}{dx^2} + [\psi_p - p\phi_p - 3\phi - \frac{(n-2)(\phi + p\psi)}{1+p^2}] \left(\frac{d^2y}{dx^2}\right)^2 \quad (3)$$

The relation between k and n is

$$n = \frac{2}{k+1}. \quad (4)$$

The system S_k is undefined for $k = -1$ or $n = \infty$.

The important systems of physical interest are:

The system S_0 of *trajectories* defined by $k = 0$ or $n = 2$.

The system S_1 of *general catenaries* defined by $k = 1$ or $n = 1$.

The system S_{-2} of *general brachistochrones* defined by $k = -2$ or $n = -2$.

The system S_∞ of *velocity curves* defined by $k = \infty$ or $n = 0$.

3. Every system S_k of ∞^3 curves is of the type (G), that is, it is defined by a differential equation of third order of the form

$$\frac{d^3y}{dx^3} = G(x, y, p) \frac{d^2y}{dx^2} + H(x, y, p) \left(\frac{d^2y}{dx^2}\right)^2. \quad (5)$$

Conversely we have proved that *except for the velocity systems S_∞ , any family of ∞^3 curves of the type (G) always represents a system S_k in a generalized field of force. There are $\infty^{(2)}$ such fields of force.*

On the other hand, we have shown that a family of curves of the type (G) is a velocity system S_∞ if and only if it is a system of curvature trajectories.

Thus the differential equation of third order defining any velocity system S_∞ is

$$\frac{d^3y}{dx^3} = (\alpha_x + p\alpha_y) \frac{d^2y}{dx^2} + \alpha_p \left(\frac{d^2y}{dx^2}\right)^2. \quad (6)$$

This system of ∞^3 curves consists of the curvature trajectories of the family of ∞^2 curves: $d^2y/dx^2 = e^{\alpha(x, y, p)}$.

For the positional case, the functions G and H need to be specialized further. A characteristic set of *five geometric properties* for the system S_k in positional fields of force have been obtained by Kasner.³

4. We shall define the angular rate λ which is associated with any lineal-element E of the plane for the motion of a particle when acted upon by a generalized field of force. As the lineal-element E rotates about its point P , the corresponding force vector F also rotates about P . The *angular rate* λ is the instantaneous rate of change of the inclination of F with respect to the inclination of E . It is given by the formula

$$\lambda = \frac{(1 + p^2)(\phi\psi_p - \psi\phi_p)}{\phi^2 + \psi^2}. \quad (7)$$

For any ordinary positional field of force, $\lambda = 0$. However, the angular rate λ vanishes not only for positional fields but also for those generalized fields of force such that the direction of the force vector at any point P does not vary but its magnitude does.

The possible *lines of force* are defined by

$$\psi(x, y, p) - p\phi(x, y, p) = 0. \quad (8)$$

Along any line of force, the angular rate λ is

$$\lambda = \frac{\psi_p - p\phi_p}{\phi}. \quad (9)$$

If there exists a lineal-element E_0 which satisfies (8) and if $\lambda \neq 1$ at E_0 , then (8) can be solved for p in the neighborhood of E_0 , that is, $p = f(x, y)$. In general, this has ∞^1 integral curves, namely, the lines of force.

5. At a point P , there is, in general, a *single particular curve* C_0 of the system S_k such that the particle describing this curve C_0 has zero speed at P . The direction of C_0 at P coincides with that of the force vector. Conversely there are ∞^1 curves of the system S_k in the direction of the force vector. The particle describing each one of these curves except C_0 have speeds different from zero at P ; but then the curvatures are zero at P . For the particular curve C_0 , the speed of the particle at P is zero but its curvature at P is *not* zero in general. For the system S_0 of trajectories, the curve C_0 is the rest trajectory. In any system S_k , there are ∞^2 such curves C_0 .

The ratio ρ of the curvature of the particular curve C_0 to the curvature of the tangent line of force, is

$$\rho = \frac{1 - \lambda}{1 + n - \lambda} = \frac{(1 + k)(1 - \lambda)}{(3 + k) - (1 + k)\lambda}. \quad (10)$$

For the system S_0 of trajectories, this is $\rho = (1 - \lambda)/(3 - \lambda)$.

For the system S_1 of catenaries, this is $\rho = (1 - \lambda)/(2 - \lambda)$.

For the system S_{-2} of brachistochrones, this is $\rho = (\lambda - 1)/(\lambda + 1)$.

For the system S of velocity curves, this is $\rho = 1$.

The above result generalizes various extensions of the theorem of Kasner concerning one-third of the curvatures.⁴

6. An arbitrary lineal-element transformation, not necessarily of the contact type, will send the ∞^3 curves of every system S_k (including $k = \infty$) into the ∞^3 curves of a system S_k if and only if it is an extended collineation or correlation.

In the positional case, we have proved that the only transformations

which convert every system S_k except $k = 0$ into a system S_k are the similitudes. The only appropriate transformations for the systems S_0 are the collineations (related to Appell's transformation).

* Presented to the American Mathematical Societies, 1948.

¹ Kasner, "Differential-Geometric Aspects of Dynamics," *Am. Math. Soc. Colloquium Publ.*, **3**, 1913 (1934). Also see series of papers in *Trans. Am. Math. Soc.*, **7-11**, (1906-1910).

² Kasner and De Cicco, "A Generalized Theory of Dynamical Trajectories," *Ibid.*, **54**, 23-38 (1943).

³ Kasner, "Physical Curves," *Proc. Nat. Acad. Sci.*, **33**, 246-251 (1947).

⁴ De Cicco, "Constrained Motion Upon a Surface Under a Generalized Field of Force," *Bull. Am. Math. Soc.*, **53**, 993-1001 (1947). Kasner and Mittleman, "A General Theorem on the Initial Curvatures of Dynamical Trajectories," *Proc. Nat. Acad. Sci.*, **28**, 48-52 (1942).

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*GALACTIC AND EXTRAGALACTIC STUDIES, XIX. GIANT
VARIABLE STARS OF THE LOOP NEBULA (30 DORADUS)*

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When in 1937 a large cluster of blue supergiant stars was found embedded in the heart of the 30 Doradus nebula by Shapley and Paraskevopoulos,¹ attention was called to the vast extent of the bright nebulosity that these stars excite, and also to the probability that heavy absorption prevails in the region, especially out beyond the luminous nebulosity. To test the degree and nature of this absorption, a study of the variable stars of the area was begun, and this communication reports on the results. An accidental by-product of the study—namely, the accurate determination for the first time of the absolute luminosity of giant Beta Lyrae variables—is of higher interest than the original goal.

1. The over-all diameter of the bright and dark nebulosity is something more than 30'. We have therefore chosen an area of one square degree, centered on the nebula, for special examination. In this area Miss Leavitt had already marked 38 stars as variable.² We now believe that five of them are not variable and five others are doubtful. Later Miss S. F. Mussells marked 26 additional objects in this square degree,³ of which seven are certainly variable, ten doubtful and the others probably invariable.

Of the 36 stars⁴ now definitely accepted as variable, only five lie within 15' of the nucleus and inside the visible bounds of the nebulosity; but quite possibly dark absorption may extend irregularly over a much larger region and dim the stars throughout most of the chosen square degree. Of the 36 variables, 18 are Cepheids, 4 eclipsing binaries, 11 irregular, 1 long-period, 1 semiregular and 1 possibly cluster type.

A quantitative test for absorption is provided by the Cepheid variables. A total of 18 lie within the square degree. Their magnitudes have been based on the standards in Main Sequence VI and in two subsidiary sequences set up in the course of the extended photometric studies in the Large Cloud of forty selected Cepheid variables.⁵ The magnitudes of

the 18 Cepheids in the neighborhood of the Loop Nebula are, therefore, on the same scale and zero point as those employed in determining the standard period-luminosity curve for the two Magellanic Clouds. Hence the standard curve can be used, through the agency of deviations, in testing for space absorption.

The numbers, positions, periods, maximum magnitudes and other data about all the variables of the 30 Doradus region are given in the accompanying table. Seven of the questionable stars are included, all with amplitudes of six-tenths of a magnitude or less. The periods are given in days, and the distances from the center of 30 Doradus (last column) are in minutes of arc.

The number and series of the photographic plates used for the measures have depended upon the position and brightness of each variable, and on the difficulties presented by its light curve. Some of the stars were troublesome because of overlapping star images; some because of the surrounding nebulosity. The differing background between variable and comparison star, and from plate to plate, makes the work in the 30 Doradus region comparable to work on the puzzling variables of the Orion Nebula, where many investigators during the past hundred years have been baffled by the difficulty of detecting regularity in the magnitude variations. In both the Orion and the 30 Doradus nebulosities many stars are suspected, and then dropped as unproved; the suspicion of variability is in part a consequence of the uneven nebulous background.

The proved variables around 30 Doradus, other than Cepheids, belong predominantly to the irregular class. The color of the variables has been determined only qualitatively from the intercomparison of red and blue plates made on the same night with the Bruce telescope with exposures of 120 minutes and 30 minutes, respectively. No dependable red magnitude standards are available for the Clouds. HV 2763 and HV 6002 were at minimum when the photographs were made, and in consequence below the plate limits.

The maximum magnitudes of all stars in table 1 range from 13.8 to 15.9, with a mean value of 15.06, which corresponds to absolute photographic magnitude -2.2 in the Cloud, with a spread from -3.4 to -1.3 . The conspicuously red bright variables, like HV 2669, 2679, 2730, and 2798, if Cloud members, as is highly probable, must have absolute visual magnitudes in excess of -5 , and therefore are even brighter than Betelgeux, which also is a red irregular variable.

The positions of the variables in table 1 are shown in figure 1, which is reproduced from a Bruce plate. The seven stars for which the type of variability could not be confirmed are not plotted. The following stars, all within the square degree, are now not considered appreciably variable on the basis of new measures of Bruce plates: HV 1015, 2693, 2753, 2755,

TABLE I

RV	X"	Y"	TYPE	PERIOD	MAX.	RANGE	MEDIAN	COLOR	R'
2669	14845	8846	Irreg.?	14.3	0.5	...	Red	32
2674	14937	9572	Irreg.	14.6	0.7	...	Red	26
5938	15042	11238	Cepheid	4.1743	15.5	0.8	15.9	..	33
2677	15066	10324	Irreg.	15.0	1.5	...	Red	25
2679	15108	8926	Irreg.	14.3	0.6	...	Red	28
5940	15148	9080	Cepheid	2.36993	15.9	0.7	16.25	..	26
2681	15156	9336	Irreg.	14.6	0.6	...	Red	24
2685	15232	11364	Cepheid	6.5415	14.86	1.08	15.40	..	33
2687	15260	10930	Semi-reg.	73:	14.9	0.6	27
2691	15420	10674	Eclips.	1.2555	15.25	0.55	23
11088	15442	9994	Irreg.	15.3	0.7	...	Red	18
5954	15461	8160	Cepheid	6.13595	15.51	0.56	15.79	..	33
2697	15467	11055	Cepheid	3.61885	15.2	1.1	15.75	..	26
1005	15689	10884	Cepheid	18.70976	13.96	1.46	14.69	..	22
1006	16035	8474	Cepheid	14.21141	14.25	1.50	15.00	..	24
2728	16113	8449	Irreg.	14.5	1.1	...	Red	24
2730	16153	8714	Irreg.?	14.2	0.6	...	Red	20
2732	16269	8484	Irreg.?	14.5	0.6	...	Red	23
2740	16354	9601	Irreg.	15.0	1.5	...	Red	5
2744	16563	8947	Eclips.	15.2	0.5	15
2749†	16654	9021	Cepheid	23.0	15.2	0.9	15.65	..	14
5989*	16788	8130	Eclips.	1.3069	15.25	0.50	29
5987	16796	9910	?	14.8	0.5	5
2761	16815	9024	Irreg.?	15.3	0.5	...	Red	15
2763	16837	8066	L. Per.	400	14.5	>2.5	30
2765	16864	9261	Clust.?	14.5	0.8	12
2773	17137	10945	Cepheid	6.34900	15.03	1.05	15.55	..	21
2774	17144	11038	Eclips.	3.65242	15.1	0.6	23
2776	17156	10864	Irreg.?	15.3	0.5	...	Red	20
1017	17207	9064	Irreg.	14.9	1.1	...	Red	17
2779	17225	11146	Cepheid	5.3217	15.25	0.75	15.62	..	25
2781	17254	9143	Irreg.	14.7	0.9	...	Red	17
2787	17324	9946	Cepheid	11.442	14.48	1.03	14.99	..	15
6004	17339	10920	Cepheid	4.3704	15.9	0.7	16.25	..	24
2790*	17381	9588	Cepheid	6.60615	15.56	0.90	16.01	..	15
6002*	17484	9468	Irreg.	15.9	>1.1	17
2793	17574	11304	Cepheid	19.1843	13.8	1.5	14.55	..	30
2795	17675	10752	Cepheid	3.91332	15.48	1.1	16.03	..	24
2798	17761	9629	Irreg.	14.3	0.7	...	Red	21
6018	17880	8453	Cepheid	4.7833	15.7	0.6	16.0	..	33
2814	18245	9381	Cepheid	5.20870	15.2	0.9	15.65	..	30
6013*	18258	10668	?	15.8	0.4	32
2815	18264	9033	Cepheid	11.98	14.4	0.9	14.85	..	33

* Positions recomputed.

† Period determined from 110 observations for years 1938 to 1946 is 23.1139 days.

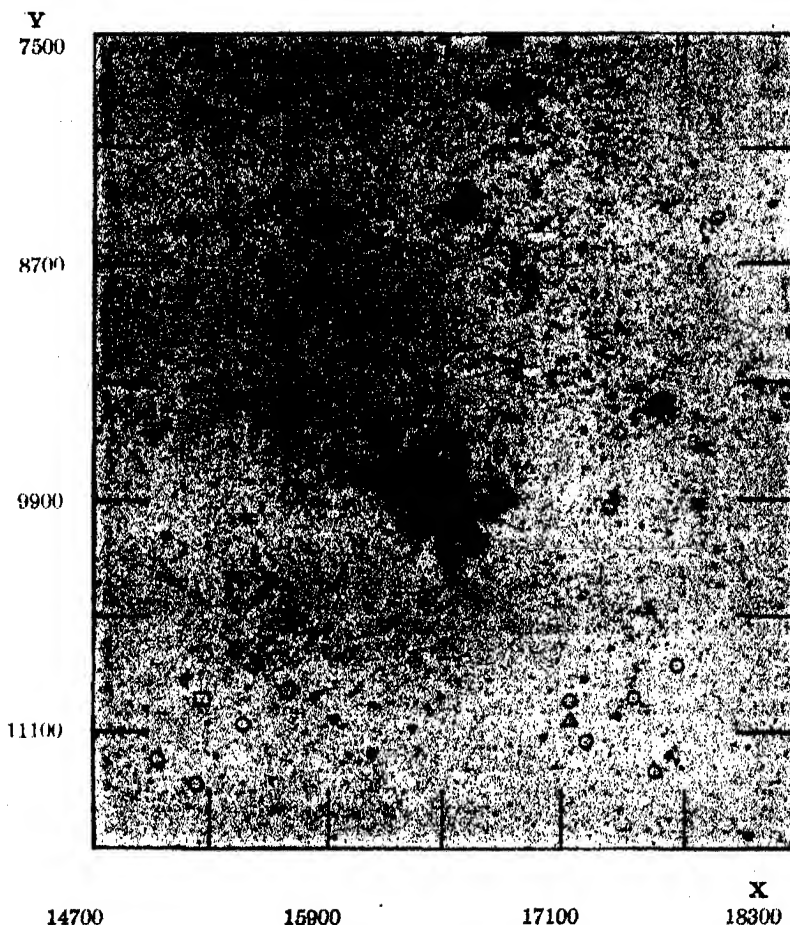


FIGURE 1

Negative of 30 Doradus. Variable stars are indicated as follows: open circles, Cepheids; triangles, eclipsing stars; squares, long-period and semi-regular; arrow heads, irregular; broken square, possibly cluster-type. Coordinate system in seconds of arc. The top 10' strip on this photograph does not enter the investigation.

2770, 5930, 5931, 5935, 5950, 5952, 5955, 5960, 5968, 5970, 5993, 5994, 5999, 6005, 6007, 6014, 6021, 6024. Their considerable number again emphasizes the difficulty of variable star photometry in regions of irregular nebulosity.

2. Returning to the question of absorption, we plot in figure 2 the apparent median magnitude against the logarithm of the period for the 18 Cepheid variables. The straight line

$$\bar{m} = 17.14 - 2.08 \log P$$

where \bar{m} is the apparent median magnitude and P the period in days, is the standard period-luminosity curve for the Large Cloud. The algebraic mean of the deviations in magnitude is $O - C = +0.18$; or, excluding HV 2749, it is $+0.12$. On the average, the absorption produced by the 30 Doradus nebulosity on these Cepheid variables is probably not more than one- or two-tenths of a magnitude. The outstanding indication of absorption is for HV 2749, for which $O - C = +1.34$. The star is nearest of all Cepheids to the nucleus of the nebula.⁶

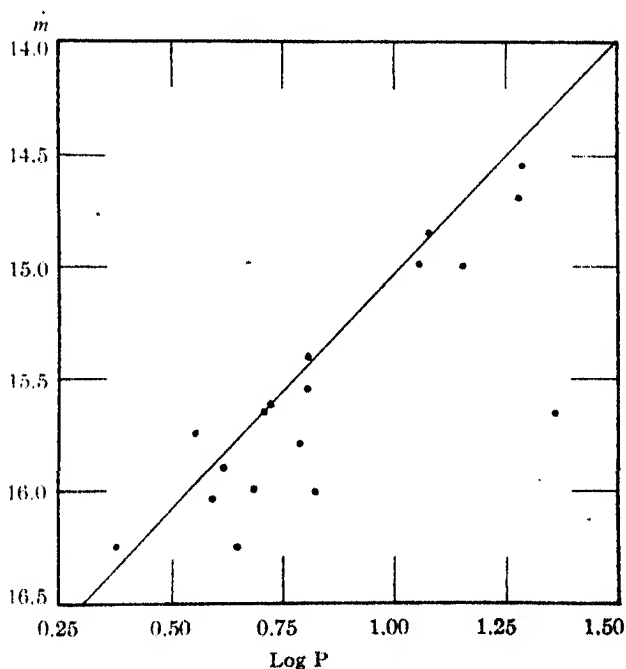


FIGURE 2

Deviations from standard period-luminosity curve indicate absorption by 30 Doradus nebulosity.

3. The four eclipsing binaries in the 30 Doradus square degree are remarkably similar:

HV	PERIOD, <i>d</i>	MAXIMUM, <i>m</i>	PRIMARY, <i>m</i>	SECONDARY, <i>m</i>
2891	1.2555	15.25	0.55	0.25
5989	1.3069	15.25	0.5	0.40
2774	3.65242	15.1	0.6	0.20
2744	15.2	0.5	0.1:

The first three belong to the Beta Lyrae sub-class. For HV 2744 only four minima have been observed; the period may be related to 1.922 days. The chances are very good that all four eclipsing stars are members of the Magellanic Cloud and therefore are giant blue binaries with photographic absolute magnitude at maximum of approximately -2 . This Cloud membership is made highly probable through a study of the frequency of faint variable stars in neighboring regions outside the Cloud—in VSF 212, 524 and 566.⁷ One eclipsing binary for each ten square degrees is the find in these comparison regions where comparable magnitude intervals enter the systematic survey. In the 200 square degrees there is in fact no eclipsing variable fainter than magnitude 14.9. The finding of four 15th-magnitude eclipsing stars in one square degree around 30 Doradus can therefore only mean membership in the Cloud.

In an earlier study of the eclipsing binaries in the fields of the Large and Small Magellanic Clouds,⁸ we suggested that the stars were probably superposed members of our own galactic system. The further study, however, of the frequency of eclipsing stars in comparable galactic latitudes has now convinced us that the eclipsing stars of the 30 Doradus region, and almost all of the score of eclipsing variables found on the plates of the Small Magellanic Cloud, are actually giant eclipsing binaries that are physical members of these nearest of external galaxies. The mean maximum magnitude of six Beta Lyrae stars in the Small Cloud, for which periods and light curves have been determined, is 15.13 ± 0.25 (m.e.), again corresponding to absolute magnitude -2 , approximately.

A further study of the Small Cloud's eclipsing stars will be undertaken. It begins to appear that the Magellanic Clouds, which have heretofore in other respects been important tools for the study of various problems, can now yield our most reliable information on the absolute magnitudes of giant eclipsing binaries. Eventually they may reveal fainter eclipsing stars. Spectroscopic observations can later be made on these bright binaries; but at the moment we must report that spectra are unavailable for any of the 36 variables of the 30 Doradus region.

The very close similarity in magnitude and range of these bright binaries near 30 Doradus is reminiscent of the essentially identical magnitudes, ranges and periods of three long-period variables in the globular cluster 47 Tucanae.⁹ In the Milky Way, eclipsing stars show large dispersions in periods, amplitudes and presumably in absolute magnitude at maximum.

4. In summary, we set out to test the degree of light absorption in the great 30 Doradus nebula of the Large Magellanic Cloud but ended with no very positive contribution to the solution of that problem. But three by-products are of considerable interest. The first is that 18 Cepheids in one square degree provide a good representation of the total Cepheid population in period-distribution, amplitudes, and relation of

luminosity to period. The second is that in this region of chaotic diffuse nebulosity, irregular variation in the light of deeply red stars is prevalent. And, of most significance, the absolute photographic luminosities of giant eclipsing binary stars are determined with relatively high accuracy, and a preference for absolute magnitude -2 is indicated.

¹ *Astrophys. J.*, **89**, 340-342 (1937); *Harvard Reprint* 141; see also *Harv. Circ.* 271, 8, 1925.

² *Harv. Ann.*, **60**, No. 4, 87-108 (1908).

³ *Ibid.*, **90**, No. 1, 1-25 (1933).

⁴ A star only suspected of variability by Miss Leavitt now proves to be conspicuously variable and has been assigned the number HV 11088.

⁵ *Harv. Ann.*, **90**, No. 10, 253-261 (1940); These PROCEEDINGS, **26**, 326-332 (1940); *Harvard Reprint* 202.

⁶ These PROCEEDINGS, **26**, 541-548 (1940); *Harvard Reprint* 207; for HV 2749 see D. Hoffleit, *Harv. Bull.* 905, 25, 1937.

⁷ *Harv. Ann.*, **90**, 171 (1934); *Harv. Bull.* 917, 1-5 (1943).

⁸ *Harv. Bull.*, 916, 19-20 (1942).

⁹ These PROCEEDINGS, **27**, 440-445 (1941); *Harvard Reprint* 228.

A NEW THEORY OF SECONDARY NON-DISJUNCTION IN FEMALE *DROSOPHILA MELANOGASTER**

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Sturtevant and Beadle¹⁶ have shown that female exceptions constitute some 36.6% and 45.6%, respectively, of the female classes derived from *Drosophila melanogaster* mothers heterozygous for *In(1)C* or *In(1)dl-49* and possessing a *Y*-chromosome. The female "exceptions" are so-called because they derive both their *X*-chromosomes from their *XXY* mothers, not one *X*-chromosome from each parent as is usually the case. This anomalous situation is brought about by a failure of segregation, that is, by "secondary non-disjunction,"¹ of the *X*-chromosomes in a percentage of the oöcytic meioses, and is greatly enhanced by the presence of heterozygous *X*-chromosome inversions along with the *Y*-chromosome as Sturtevant and Beadle have demonstrated.

The frequency of non-disjunction is not equal to the frequency of exceptional females among the female class, however, for only half of the fertilized *XX* non-disjunctional eggs ordinarily emerge as adult flies, namely, the half that is fertilized by *Y*-bearing spermatozoa. The other half that is fertilized by *X*-bearing spermatozoa gives rise to superfemales having three *X*-chromosomes, and under ordinary conditions superfemales

only rarely eclose from their puparia.^{9, 12} Accordingly, if p represents the percentage of secondary non-disjunction, or, better $(XX)-(Y)$ segregation, and q the frequency of exceptional females, then

$$p = 100(2q/1 + q).$$

From this relation it follows that about 54% of all segregations in $\text{In}(1)C/+Y$ are non-disjunctive for the X -chromosomes, and some 63% of all segregations in $\text{In}(1)dl-49/+Y$ females are also of the type $(XX)-(Y)$. The high frequencies of secondary non-disjunction in these two cases are of more than passing significance, for as Sturtevant and Beadle¹⁶ remark, they are in sharp disagreement with predictions based upon Bridges' generally accepted and now classic hypothesis concerning the mechanism of secondary non-disjunction.

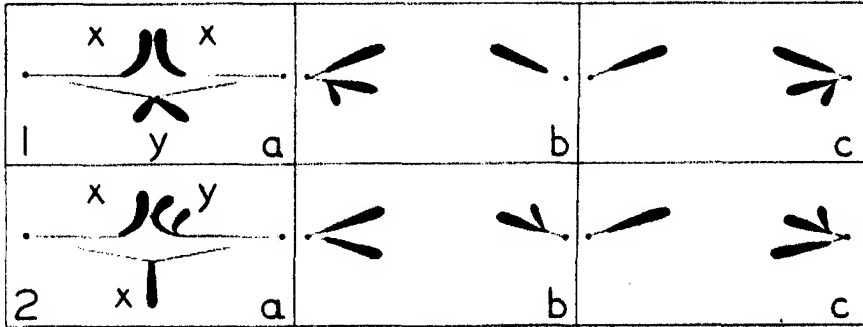


FIGURE 1

Purely diagrammatic representation of Bridges' (1916) hypothesis of secondary non-disjunction. Line 1, the consequences of XX -conjunction, Y being excluded from association. Line 2, the consequences of XY -conjunction, one X being excluded from association. Equally probable anaphase patterns arising from the particular metaphase associations are represented in blocks b and c . Secondary non-disjunction has a maximum value of 50% on this hypothesis.

Bridges' (1916)⁸ hypothesis was in total accord with all of the known facts at the time at which it was proposed, and remained a satisfactory accounting of the mechanism and consequences of secondary non-disjunction until Sturtevant and Beadle's work upon heterozygous X -chromosome inversions. Bridges assumed that in XXY females the two X -chromosomes and the Y -chromosome are in mutual competition for a pairing or conjunctive partner, and that when any two of these three homologous chromosomes succeed in pairing, the third is left unassociated. Furthermore, the univalent chromosome was supposed to distribute randomly with respect to the poles, as is known to be the case for many univalents. The operation of the hypothesis is diagrammatically represented in figure 1. Line 1 represents the consequences of XX -pairing,

leaving Y a univalent to assort randomly. Line 2 represents the non-disjunctive process as Bridges conceived it. One X pairs with Y , the other X being a univalent. When anaphase occurs the univalent X goes to the Y -pole (2c) in 50% of the cases, and to the same pole of the spindle as the other X (2b) in the remaining 50% to give XX non-disjunctional nuclei. On Bridges' hypothesis, therefore, one hundred per cent pairing of Y with an X , could it be brought about, would give a maximum p of only 50%, and a maximum q of 0.33 as the frequency of exceptions among the female class of offspring. Thus the 54% non-disjunction found in $\text{In}(1)C/+Y$ and 63% for $\text{In}(1)dl-49/+Y$ cannot be accounted for on the basis of Bridges' assumptions. As Sturtevant and Beadle^{16, 17} remark, a new interpretation of secondary non-disjunction must be sought as "the general problem of the mechanism of secondary non-disjunction is unsolved." It is the purpose of this note to provide such a new interpretation of the mechanism of secondary non-disjunction, along with data on several synthetic high non-disjunctional lines upon which the new interpretation is based.

All chromosomes (including the Y) of the *Drosophila melanogaster* stocks employed in the research now reported were derived from *Canton-S*, with the exception of the inverted X -chromosomes and the possible exception of the fourth chromosomes in some strains. Special methods were adopted to guarantee that homologous large autosomes were isosequential through all the stocks employed, and this was also the case for any non-inverted X -chromosome used in the crosses. The reason for building up such special stocks, the chromosomes of which are isosequential *inter se*, lies in Sturtevant's¹⁴ notable discovery that heterozygous inversions in the large autosomes may markedly *decrease* rates of secondary non-disjunction in X -chromosome structural heterozygotes. Experiments on comparative rates of secondary non-disjunction may be expected to gain in sensitivity, to be more reproducible, and more revealing of unique qualities (if any) of inversions, therefore, when such potentially disturbing factors as autosomal structural (=sequential) heterozygosity are removed.²

The effects upon secondary non-disjunction of heterozygous combinations of $\text{In}(1)B^{M1}$, $\text{In}(1)dl-49$, and $\text{In}(1)AM$ with wild-type (+) X -sequences, and in certain combinations with one another, are given for the female classes in table 1. (Data for the male classes are in good agreement with those for the female progeny, but are not recorded here as they require corrections for exchanges within inversions, as well as for viability differences in some cases.) $\text{In}(1)B^{M1}$ is roughly ten map units long, and has its left break just to the right of B (57.0), and its right break probably to the right of bb (66.0). $\text{In}(1)dl-49$ is perhaps thirty map units long, having its left break between rb and cv (i.e., between 7.5 and 13.7) and its right break between fw and g (between 38.3 and 44.4). $\text{In}(1)AM$ is also about thirty

TABLE 1

THE PERCENTAGES OF EXCEPTIONAL FEMALES AND OF SECONDARY NON-DISJUNCTION IN *Drosophila melanogaster* MOTHERS HETEROZYGOUS FOR THE SPECIFIED INVERSIONS AND HAVING THE Y-CHROMOSOME AND ISOSEQUENTIAL AUTOSOMES DERIVED FROM Canton-S. THE STANDARD OR WILD TYPE SEQUENCE FOR THE X-CHROMOSOME IS REPRESENTED BY "+"

CONSTITUTION OF MOTHER	TOTAL FEMALE OFFSPRING	EXCEPTIONAL FEMALES, %	(XX)-(Y) SEGREGATIONS, %
+ / + / Y (= control)	2121	0.9	2
In(1)B ^{M1} / + / Y	2465	8	14
In(1)dl-49 / + / Y	3705	54	70
In(1)AM / + / Y	2277	54	70
In(1)dl-49 / In(1)B ^{M1} / Y	1981	61	76
Ins(1)dl-49, B ^{M1} / + / Y	5330	63	77
In(1)dl-49 / In(1)AM / Y	2171	67	80

TABLE 2

THE OCCURRENCE OF EXCEPTIONAL FEMALES BY PRIMARY NON-DISJUNCTION IN *Drosophila melanogaster* MOTHERS HETEROZYGOUS FOR SPECIFIED INVERSIONS AND HAVING THE AUTOSOMES ISOSEQUENTIAL AND DERIVED FROM Canton-S. THE STANDARD OR WILD TYPE SEQUENCE FOR THE X-CHROMOSOME IS REPRESENTED BY "+." COMPARE WITH TABLE 1

CONSTITUTION OF MOTHER	TOTAL FEMALE OFFSPRING	TOTAL EXCEPTIONAL FEMALES	(XX)-(O) SEGREGATIONS, %
+ / + (= control)	8081	1	0.02
In(1)B ^{M1} / +	2660	0	..
In(1)dl-49 / +	3155	10	0.6
In(1)AM / +	1133	1	0.2
In(1)dl-49 / In(1)B ^{M1}	1389	4	0.6
Ins(1)dl-49, B ^{M1} / +	5613	10	0.4
In(1)dl-49 / In(1)AM	1159	0	..

map units in length, extending from near l_2 (27.7) to between B and Bx (i.e., between 57.0 and 59.4).⁵ It is seen that the inversion heterozygotes markedly differ in non-disjunctional rates from the control, and that secondary non-disjunction sharply rises with an increase in the structural difference existent between the two X-chromosomes. Furthermore, excepting the cases of + / + / Y and B^{M1} / + / Y, none of the non-disjunctional rates found are interpretable upon the basis of the classical hypothesis which sets 50% as the uppermost limit of secondary non-disjunction.

Table 2 records the occurrence of primary exceptions among the offspring of females having X-chromosome constitutions identical with those of table 1, but lacking a Y-chromosome. As Sturtevant and Beadle¹⁶ concluded from their own data, and as table 2 shows, mere heterozygosity for X-chromosome inversions has but a negligible effect upon the final outcome and regularity of X-chromosome segregation in female *Drosophila melanogaster*. Thus very high non-disjunction rates depend not only upon

the particular combination of heterozygous inversions present, but in addition *require* the presence of a *Y*-chromosome. There is no doubt that Bridges was correct in his opinion that the *Y*-chromosome somehow brings about non-disjunction by its own segregative involvements.

Now it is known that the presence of these inversions in heterozygous combinations markedly increases the proportion of non-exchange tetrads formed by the *X*-chromosomes,^{10, 12, 16, 19} whether or not a *Y*-chromosome is present. Indeed, as a conservative estimate, at least 76% of the *X*-chromosome pairs in *Ins(1)dl-49*, *B^{w1}/+*, *Dp(1; 1)112* are non-exchange tetrads,¹⁰ and this is also the case where the uninverted chromosome lacks a duplication. The degree to which crossing-over is eliminated may be taken as one measure of the meiotic dissimilarity of the *X*-chromosomes caused by their sequential non-homology in principally euchromatic lengths.

But as the *X*-chromosomes become sequentially dissimilar through the introduction of heterozygous combinations of inversions affecting their euchromatic lengths, so they become effectively more like *Y*-chromosomes in their pairing affinities. A moment's reflection will bring conviction that were the euchromatic lengths of two *X*-chromosomes to be made wholly dissimilar, but the so-called "inert" or chromocentral regions to remain essentially unaltered, then conjugation between the *X*-chromosomes would be predominantly a heterochromatic affair—a process occurring almost exclusively between the chromocentral regions.⁴ This, however, is merely another way of stating that conjunction becomes more frequent in those regions of *X* wherein pairing with *Y* normally occurs. Hence, as is found to be the case, when a *Y*-chromosome is present non-disjunction markedly rises in *X*-chromosome inversion heterozygotes, probably because the sequential differences between the *X*-chromosomes in effect emphasize their likenesses to the *Y*-chromosome. Thus, where the *X*-chromosomes are everywhere in sequential homology, *Y* induces a rise in non-disjunction from 0.02% in *+/+* to a mere 1.6% in *+/+/Y*. In other words the *X*-chromosomes share more similarities with each other than they do with the *Y*-chromosome, and *Y* becomes an effective partner to *X* in only a small proportion of the cases—perhaps less than 2% of all associations in *+/+/Y*. But where there is a great reduction in sequential homology between the two *X*-chromosomes, as in the combination *In(1)dl-49/In(1)AM*, non-disjunction rises from an indeterminately small fraction in *dl-49/AM* to about 80% when merely a *Y*-chromosome is added to the karyotype. *Y*, in other words, is a more attractive partner to either *X*-chromosome in this combination than either *X*-chromosome is to the other. The apparent paradox that each *X* in effect finds *Y* a more attractive and frequent conjunctive partner than the other *X*-chromosome, although the *X*-chromosomes remain alike in the very regions with which

they share homology with Y , is resolved by the assumption that *both arms* of the Y -chromosome share homology and may thus conjoin with an X .⁶ Hence if one X pairs with one arm of Y , then the other X must conjoin with the other arm of Y if it is to have a meiotic partner at all. The mechanical aspects of this mode of bringing about an apparent non-disjunction by trivalent formation of both X -chromosomes with a Y -chromosome are diagrammed in figure 2. Since, for example, X_a pairs with arm Y_a , and X_b with Y_b , the segregation patterns are $X_a - Y_a$ and $X_b - Y_b$. But in an alternately oriented trivalent Y_a and Y_b perforce must go to one pole, since they possess the same kinetochore, and X_a and X_b undergo forced segregation to the other pole since each disjoins from Y .

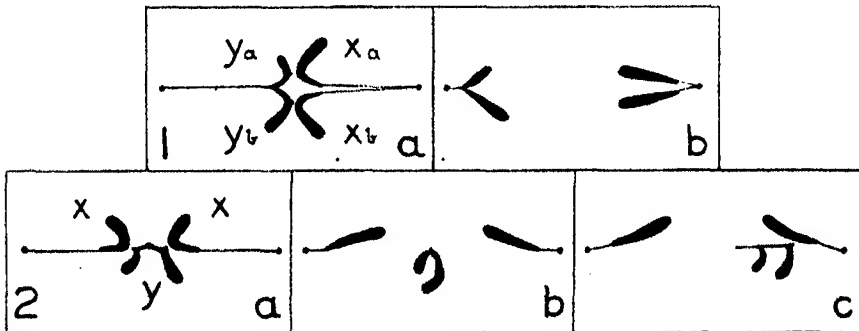


FIGURE 2

Purely diagrammatic representation of the interpretation of secondary non-disjunction proposed here. Line 1 of figure 1 is held to represent accurately the consequences of XX -conjunction in XXY females. Line 1 of figure 2 shows an XYX trivalent and the imposed non-random segregation following alternate coorientation of the associated chromosomes. Line 2 represents a linear orientation of an XYX trivalent, and two of the possible consequences in anaphase. Such misoriented trivalents may lead to the loss of Y and other irregularities, but do not give rise to exceptional gametes. The majority of trivalents are supposed to orient alternately as in 1a, hence this interpretation admits a possible maximum of 100% secondary non-disjunction.

The interpretation of secondary non-disjunction that is offered here asserts, therefore, that secondary non-disjunction is not a case of non-disjunction at all, but an example of imposed non-random segregation from a sex-chromosome trivalent.⁷ The mechanism proposed has a direct analogy with the segregative mechanism normally occurring for X_1 , X_2 and Y in the spermatogenesis of *Drosophila miranda*.¹¹ It may be pointed out that XYX trivalents formed at meiosis in any XXY female of *Drosophila melanogaster* may be greater than the percentage of secondary non-disjunction owing to the probable occurrence of some linear orientations of the trivalent at first metaphase (Fig. 2, line 2).

The principal virtue of this new interpretation of secondary non-disjunction lies not alone in the fact that it wholly accounts for data not

resolvable by Bridges' hypothesis, but that it can, if false, be disproved by a direct test of its predictions. The scheme proposed predicts that (1) secondary non-disjunction must rise on the average as the euchromatic sequences of the two *X*-chromosomes become increasingly different; (2) the presence of two *Y*-chromosomes should decrease the rate of secondary non-disjunction; (3) either the long arm or short arm of *Y* alone could not regularly give greater than 50% secondary non-disjunction; and (4) that in those cases where secondary non-disjunction exceeds 50%, the *X*-chromosomes must have a partner that can pair with both *X*-chromosomes simultaneously (i.e., in general the partner must have two arms). And so forth.

Each of these implications has been put to test, and each has so far proved to be in accord with the experimental facts. For example, (1) tests of $+/+/Y$, $\text{In}(1)sc^7/+/Y$, $\text{In}(1)B^{M1}/+/Y$, $\text{In}(1)sc^7/\text{In}(1)B^{M1}/Y$ and $\text{Ins}(1)sc^7, B^{M1}/\text{In}(1)dl-49/Y$ give as secondary non-disjunction rates: 2%, 14%, 14%, 63% and 78%, respectively. This is the very order that would be predicted, and since $\text{In}(1)sc^7$ and $\text{In}(1)B^{M1}$ are of the same order of size, close similarity in their effects on raising the secondary non-disjunction rate is to be expected.

(2) On the new interpretation, if each arm of *Y* is equivalent to *X* in its pairing, and if the order of pairing is random, but pairing complete in so far as possible, then in *XXY* females secondary non-disjunction would be expected to approximate a *p* of 80%. It is interesting to note that this is the uppermost value for secondary non-disjunction so far attained in my experiments. In *XXYY* females tetravalents may be expected if corresponding arms of *Y* may pair, but their characteristics of orientation is an unknown factor. However, it may be assumed that, for necessity of spatial economy on the spindle, alternate orientations (N) should exceed adjacent (M). If results for equality of these two classes of orientation be calculated, as well as for total alternate orientation, then the expectation is that secondary non-disjunction in *XXYY* females will have a *p* with some value in the interval from 27 to 52%. If it be assumed that tetravalents orient wholly at random, then *p* should approximate 52%. Should it be the case that tetravalents never form, then *p* should approximate 42%. The three possibilities treated above cover the more probable conjectures about conjunction in *XXYY* females, and the calculated values are closely similar to or include the experimental value of 36% obtained from $\text{Ins}(1)dl-49, B^{M1}/+/Y/Y$.⁸ In the control, $\text{Ins}(1)dl-49, B^{M1}/+/Y$, *p* is 78%.

(3) An effectively one-armed *Y^s*-chromosome obtained through the kindness of Professor H. J. Muller gives about 24% non-disjunction in $\text{Ins}(1)dl-49, B^{M1}/+/Y^s$ whereas the control crosses of $\text{Ins}(1)dl-49, B^{M1}/+/Y$ give 77%. (The *Y^s*-chromosome has not yet been gotten into

a background of *Canton-S* autosomes, so this test with Y^s may have given a somewhat low value. Internal evidence supplied by the controls, which are XXY daughters of the XXY^s females, suggests, however, that the true effect of the Y^s -chromosome on secondary non-disjunction in this X -chromosome karyotype will not be found to differ appreciably from the low value so far obtained.)

(4) All Y -chromosomes in the experiments undertaken which give non-disjunctional rates over 50% (with heterozygous $Ins(1)dl-49$, B^{M1}) have two effective arms. These Y -chromosomes include Y^{cs} , Y^{sw-b} , $sc \cdot Y^L$, and others.

The interpretation here posed, therefore, is in agreement with a wide variety of new facts, as well as with those past discoveries which make up the body of available data on secondary non-disjunction in *Drosophila melanogaster* females.

Summary.—A new theory of secondary non-disjunction in female *Drosophila melanogaster* is proposed. It asserts that the process is actually a case of imposed non-random segregation from an XYX trivalent. Each arm of Y is assumed to pair with a different X -chromosome to form a trivalent which usually orients in alternate pattern at first meiotic metaphase. Thus the cytological features are envisioned as similar to the directed segregation from X_1YX_2 trivalents in the spermatogenesis of *Drosophila miranda*. Previously known facts, as well as results of genetic experiments designed to test the scheme, appear to be in full accord with this interpretation.

* The research reported here was first undertaken in 1945 during tenure of a John Simon Guggenheim Memorial Foundation Fellowship at the Kerckhoff Biological Laboratories of the California Institute of Technology. The experiments have continued uninterruptedly since then in the Biological Laboratories of Princeton University and, during the summers of 1946 and 1947, at the Marine Biological Laboratory, Woods Hole, Massachusetts.

¹ Failure of the two X -chromosomes to segregate to opposite poles in the oögenesis of an XX -female is referred to as "*primary* non-disjunction." Inclusion of both X -chromosomes in a single polar group at meiosis of an XXY -female is referred to as "*secondary* non-disjunction." The distinction is an important one, for the mechanisms of primary and secondary non-disjunction are wholly dissimilar, the latter being dependent upon the extra Y -chromosome.

² Sturtevant and Beadle¹⁸ did not deliberately clear their stocks of possible sequential disturbances of the autosomes, for no such effect of autosomal inversions upon secondary non-disjunction had been anticipated in 1936. This may well account for the significantly lower rate (63%) found by them than that (70%) reported here for secondary non-disjunction in $Ins(1)dl-49/+ / Y$. Cf. table 1.

³ Additional information on these inversions can be had from: Bridges, C. B., *Carn. Inst. Wash. Pub. No. 552*, viii + 257 pp. (1944).

⁴ This conclusion perhaps deserves a general discussion of the nature of synapsis. Suffice it to say in this connection that the idea of specific forces between homologous genes is without proof, and is almost certainly not a necessary hypothesis.

⁵ Proof that each arm of *Y* shares homology with *X* will be found in Neuhaus's work; v. Neuhaus, M., *Genetics*, 22, 333-339 (1937).

⁶ A noteworthy outcome of these experiments, that will be reported in detail in another paper, is that, in general, flies whose eyes are $+/+$, $+/w^a$, w^a/w^a , $+/v$, v/v , v/w^a , v/car , car/car , etc., and which possess two (or more) *Y*-chromosomes beyond normal for the karyotype (i.e., $XXYY\varnothing$ and $XYYY\sigma$) have flecked and/or mottled eyes.

⁷ Bauer suggests that the union of two *Y*-chromosomes to form a *V* made possible the general elimination of $(X_1Y_1) - (X_2Y_2)$ segregations in tetraploid earwigs. A mechanism similar to the one proposed here would result by such translocation, and give principally $(X_1X_2) - (Y_1Y_2)$ segregations at spermatogenesis. v. Bauer, H., *Zeitschr. Naturforsch.*, 2b, 63-66 (1947).

⁸ Bridges, C. B., *Genetics*, 1, 1-52, 107-163 (1916).

⁹ Bridges, C. B., *Science*, 54, 252-254 (1921); *Am. Naturalist*, 56, 51-63 (1922); *ibid.*, 59, 127-137 (1925).

¹⁰ Cooper, K. W., *Genetics*, 30, 472-484 (1945).

¹¹ Cooper, K. W., *Ibid.*, 31, 181-194 (1946).

¹² Dobzhansky, Th., these PROCEEDINGS, 14, 671-675 (1928).

¹³ Gershenson, S., *Jour. Genetics*, 30, 115-125 (1935).

¹⁴ Morgan, T. H., and Sturtevant, A. H., *Carnegie Inst. Wash. Year Book*, 43, 164-165 (1944).

¹⁵ Stone, W., and Thomas, I., *Genetica*, 17, 170-184 (1935).

¹⁶ Sturtevant, A. H., and Beadle, G. W., *Genetics*, 21, 554-604 (1936).

¹⁷ Sturtevant, A. H., and Beadle, G. W., *An Introduction to Genetics*, Saunders Co., Philadelphia, 1939, pp. 1-391.

THE ORIGIN OF VOLUTIN ON THE CHROMOSOMES, ITS TRANSFER TO THE NUCLEOLUS, AND SUGGESTIONS CONCERNING THE SIGNIFICANCE OF THIS PHENOMENON*

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Volutin as a Cytological Entity.—Volutin, which is widely distributed in bacteria and fungi and is also called metachromatin, is a clearly defined cytological entity, distinguished by its ability to retain basic stains when the cells are rinsed in dilute acid. When volutin is stained with methylene blue or toluidine blue, it retains the stain after treatment with 1 per cent sulphuric acid. In addition to being "acid fast," volutin has the characteristic of staining red or purple with methylene blue or toluidine blue, in contrast to other basophilic components of the cell which stain blue with these dyes. Both metaphosphates and estersulphates retain dyes after destaining and stain red or purple with toluidine blue. Volutin is widely distributed in fungi and bacteria, while the estersulphates¹ are apparently widely distributed in higher animals. Wiame's work² strongly suggests

that the volutin in yeast cells is metaphosphate, because the addition of the phosphate to the cell increases the amount of volutin demonstrable in the cell, and extracts from these heavily stained volutin-containing cells yield reasonably large quantities of metaphosphate. There is, however, no cytological means of distinguishing between metaphosphates and estersulphates.

The present paper deals with the cytological observations on the cycle through which volutin originates on the chromosomes and is transferred to the nucleolus. A hypothesis concerning the significance of volutin is also presented. This hypothesis probably depends for its final validity upon the demonstration of metaphosphate in the animal cells, but this has not yet been achieved.

The Nucleolus of the Yeast Cell.—Lindegren³ was the first to describe the chromosomes in the vacuole of the yeast cell. Lindegren and Lindegren⁴ described the mitosis by which these chromosomes are partitioned equally to mother and daughter yeast cells. Rafalko⁵ showed that the chromosomes in the nuclear vacuole of the yeast cell are Feulgen-positive. Prior to his work, it was argued that the chromosomes were the Feulgen-positive structures in the body attached to the nuclear vacuole which Lindegren and Lindegren described as the centrosome.

Rafalko (unpublished) has identified the nucleolus as a structure, staining with light green counterstain attached to Feulgen-positive chromosomes in the nuclear vacuole of the yeast cell. We had previously described the nucleolus, but had called it "balled-up" chromosomes. Rafalko's demonstration of it as a Feulgen-negative globular structure attached to the Feulgen-positive chromosomes has disclosed its true nature. We had previously designated the cytoplasmic granules as the nucleolar equivalents, following Caspersson and Brandt's theory⁶ of their significance. However, the discovery of the true nucleolus makes this invalid. Except for the excessive size of the centrosome and its high content of desoxy-ribosenucleoprotein, the yeast cell is quite conventional. The cytoplasmic granules are the conventional mitochondria.

The Transfer of Volutin from Chromosomes to Nucleolus.—Chromosomes and nucleolus can be demonstrated with a stain specific for volutin⁷ which contains formaldehyde, as a killer and fixative, and locates the volutin in the cell. If the chromosomes are coated with volutin, they are stained purple. If the nucleolus is coated with volutin, it is likewise stained purple. As soon as either the chromosomes or the nucleolus lose their volutin, they are no longer specifically stainable. The density of the stain also gives some idea of the quantity of volutin present. The best results are obtained when the stain is adjusted to a pH of 2.5, which prevents the proteins in the cytoplasm from also becoming stained. The stain contains 4 cc. saturated aqueous toluidine blue, 20 cc. formalin and sufficient acetic

acid to bring the pH to 2.5. A loopful of cells is placed in 10 drops of the stain in a 4-inch test tube. The tube is allowed to stand overnight, centrifuged, the supernatant fluid discarded and a loopful of the sediment is mounted on a slide, covered with a cover glass, pressed down with blotting paper and sealed with paraffin.

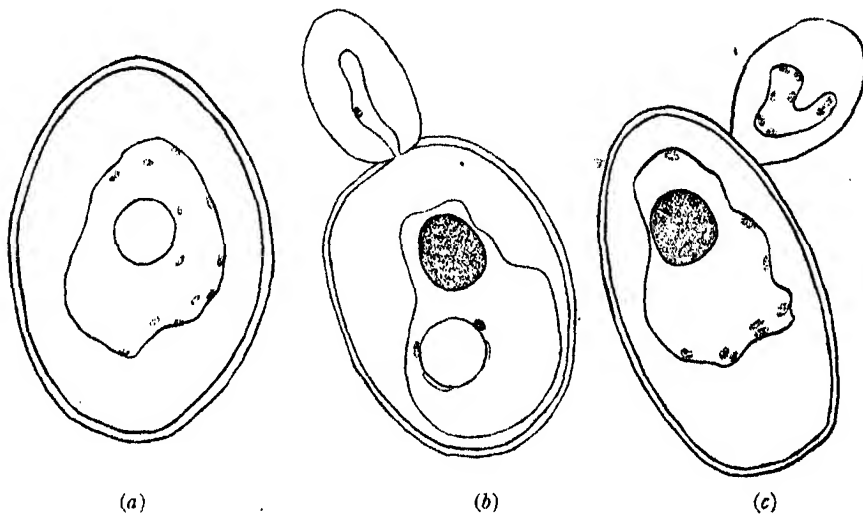


FIGURE 1

Yeast cells stained for volutin; the vacuole is shrunk by fixation. (a) A single large unstained nucleolus with many small, light pink peripheral chromosomes. (b) A budding cell with one stained and one unstained nucleolus. Two stained and two faintly stained chromosomes are attached to the unstained nucleolus. (c) A budding cell with a deeply stained nucleolus and very faintly stained peripheral chromosomes. No nucleolus visible in bud.

The growth of yeast was followed closely from resting to actively growing, vigorously fermenting cells. In resting cells, the chromosomes are unstained and the nucleolus is practically invisible. Before budding begins, the chromosomes become coated with volutin, and two unstained nucleoli become visible in each cell. As budding begins, the nucleoli increase in size, volutin disappears from the chromosomes and appears on the nucleolus. Transfer of volutin occurs by a direct attachment of *chromosome to nucleolus*, for two or three small, dark purple dots can be seen attached to an unstained nucleolus. Generally, one nucleolus becomes coated with volutin before the other, and subsequently both fuse. These fused nucleoli are the commonly described refractile "dancing bodies" in the yeast vacuole. The nucleolus is a large, round structure, possibly 2 or 3 microns in diameter. At first the interior of the nucleolus is not deeply stained with the purple dye; the color is on the outer edge or surface of

the structure. The nucleolus often becomes quite large before volutin appears on it.

The following typical results were obtained when 7 g. of compressed yeast were inoculated into 500 cc. of medium of the following composition:

Liquid yeast extract.....	6 cc.
Peptone.....	5 g.
KH ₂ PO ₄	2 g.
Corn steep water solids.....	2 g.
Cerelose.....	40 g.
per liter of tap water	

The culture was grown in a 6-liter Erlenmeyer flask on a shaker.

Yeast cells in a cake of baker's yeast are small and compact and ordinarily show little or no volutin in the vacuole. During the first hour in the nutrient broth, the cells enlarge and show a faintly staining pink volutin deposit covering the chromosomal threads. As the cells continue to swell, preparatory to division, the nuclear vacuole becomes much enlarged and the chromosomes begin to take the stain more deeply. In this stage, the shape and form of the chromosomes can be clearly distinguished, and they can be made out as single strands or pairs of small, rather compact rods. As a vacuole enlarges still more, two nucleoli also appear, but during this period, they do not stain. About the time the first buds come up, one or two nucleoli are visible as large, light green, refractile bodies in the nuclear vacuole. The chromosomes now appear as a large number of individual bodies on the inner periphery of the vacuole. After a while, one nucleolus takes on the volutin stain, followed shortly by the other, or both fuse. At the same time, the color disappears from the chromosomes or becomes very much diminished. When the bud is growing at its most rapid rate, the nucleolus is swollen and densely stained, showing that it contains a large quantity of volutin. The chromosomes are visible as small, faintly stained peripheral bodies on the inner wall of the nuclear vacuole. The nucleolus is usually visible as a stained structure only in the vacuole of the mother cell, but in some cases the vacuole of the daughter cell also contains a stained nucleolus. After division has ceased, the nucleolus retains a dense coating of volutin, but the chromosomes are unstained.

"Abnormal" Distribution of Volutin in the Yeast Cell.—Wiame² has shown that yeast cells can be starved for phosphate by growing them in a medium deficient in phosphate, such as beet molasses. When growth finally ceases, chemical analysis reveals no metaphosphate in the cells, and the cells do not contain volutin. Furthermore, the cytoplasm is colorless showing a marked diminution of basophilic protein in the cytoplasm. He centrifuges these cells, washes them in water and re-suspends them in a 2 per cent sugar solution, adding $M/30$ KH₂PO₄. Wiame kindly performed this experi-

ment with me in my laboratory and allowed me to follow the process cytologically. The chromosomes became rapidly coated with volutin, staining red by the volutin stain. Volutin sometimes appeared on the chromosomes within three minutes after the addition of the phosphate. After twenty minutes, volutin began to appear in the cytoplasm and the volutin in the vacuole decreased rapidly as the entire cytoplasm became faintly pink. Wiame's chemical tests show that there is a steady increase in the total metaphosphate in the cells and that the over-all concentration of metaphosphate in the cells continually increases in spite of the fact that volutin first appears in the chromosomes and then disappears from them and continues to accumulate later exclusively in the cytoplasm. Concurrent stains with the methylene blue dead cell stain reveal that the cells containing volutin in the cytoplasm are dead. This is consistent with the fact that metaphosphate is capable of precipitating proteins. The appearance of volutin in detectable amounts in the cytoplasm is a definite criterion of death. The rate of death can be reduced by adding the phosphate to the stained cells very slowly, but in a medium containing only 2 per cent sugar, the phosphate eventually results in the death of the cells. When normal cells (in an adequate medium) take up phosphate, the volutin stain reveals that as growth progresses, the cytoplasm becomes blue and the chromosomes red, indicating that there is basophilic protein but no stainable volutin in the cytoplasm of the healthy yeast cell. Healthy growth occurs only if a properly balanced nutrient is added slowly; phosphate alone produces a condition of imbalance which eventually results in death.

Volutin and Ribosenucleoprotein Synthesis.—The loss of stain from the chromosomes and its accumulation in the nucleolus indicate that the volutin formed on the chromosomes has been transferred to the nucleolus. Apparently, the synthesis of volutin occurs on the surface of the chromosome, and as soon as the chromosome becomes densely loaded, the transfer to the nucleolus occurs. Caspersson and Schultz⁵ have described the nucleolus of higher plants and animals as the center for the synthesis of ribosenucleoprotein. They showed that after ribosenucleoprotein is synthesized in the nucleolus in the cell of a higher plant or animal, it travels through the membrane into the cytoplasm. Caspersson and Brandt⁶ did not observe the nucleolus in the yeast cell with ultra-violet light, possibly because the cytoplasm contains so much nucleoprotein that the smaller amount in the nucleolus is overshadowed. They did show (with ultra-violet light) that ribosenucleoprotein accumulates on the outside of the nuclear vacuole. They thought it was synthesized at this point, but the demonstration of a conventional nucleolus in the nuclear vacuole suggests that the accumulation of ribosenucleoprotein, demonstrated on the surface of the nuclear membrane, may be the accumulation of this substance, as it diffuses from the nucleolus through the nuclear membrane

and into the cytoplasm. This view is in agreement with Caspersson and Schultz's ideas⁵ concerning the rôle of the nucleolus in the synthesis of ribosenucleoprotein in higher plants and animals, but Caspersson and Brandt's⁶ findings concerning the yeast cell were based on the incomplete cytological information on the yeast cell available at that time.

An Hypothesis of the Rôle of Volutin (Metaphosphate) in Conferring Specificity on Cellular Enzymes.—Wiame² has presented convincing evidence supporting the view that volutin is metaphosphate. Assuming that his theory is correct, one may develop the following hypothesis:

Metaphosphate is synthesized from orthophosphate on the chromosomes and is transferred to the nucleolus. The synthesis of ribosenucleoprotein occurring in the nucleolus depends upon a supply of metaphosphate originating in the chromosome, and the specificity of cellular enzymes may be associated with this phenomenon, since genes are known to control the specificity of various cellular enzymes. Von Euler and Janssen⁹ showed that the apoenzyme (protein) components of cellular enzymes are responsible for their specificity, and this important fact was recently confirmed by Spiegelman, Reiner and Morgan;¹⁰ Caspersson and Schultz showed that the cellular ribosenucleoproteins are synthesized in the nucleolus and transferred from the nucleolus to the cytoplasm. It is, therefore, proposed that the specificity of enzymes whose synthesis is under genetic control depends upon specific substances carried with the polymerized metaphosphate into the nucleolus.

Orthophosphate cannot be used directly in the formation of ribosenucleoprotein, but must first pass through the metaphosphate stage. The energy-rich phosphate bond in metaphosphate furnishes the energy which is required for the synthesis of the ribosenucleoprotein in the nucleolus, as well as of the proteins of the chromosome itself. The synthesis of ribosenucleoprotein occurring in the nucleolus must initiate the production of specific enzymes. This suggests that the metaphosphates carry specificity-conferring groups with them from the chromosome to the nucleolus, which give specificity to the ribosenucleoproteins synthesized in the nucleolus.

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¹ Sylvén, Bengt, *Acta. Chir. Scand.*, *Supplement*, 66, 151 pp. (1941).

² Wiame, J. M., *Fed. Proc. Am. Soc. Expt. Biol.*, 6, 302 (1947).

³ Lindegren, C. C., *Mycologia*, 47, 767-780 (1945).

⁴ Lindegren, C. C., and Lindegren, Gertrude, *Cold Spring Harbor Symposium on Quantitative Biology*, 11, 115-129 (1947).

⁵ Rafalko, J. S., *Stain Technology*, 21, 91-93 (1946).

⁶ Caspersson, T., and Brandt, K., *Protoplasma*, 35, 507-526 (1941).

⁷ Lindegren, C. C., *Nature*, 159, 63 (1947).

⁸ Caspersson, T., and Schultz, Jack, *Proc. Nat. Acad. Sci.*, 26, 507-515 (1940).

⁹ v. Euler, H., and Janssen, B., *Z. physiol. Chem.*, **169**, 226 (1927).

¹⁰ Spiegelman, S., Reiner, John M., and Morgan, Ida, *Arch. Bio.*, **13** (1), 113-125 (1947).

BOUNDARIES OF ULC SETS IN EUCLIDEAN n -SPACE

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In this note an example is given of an $(n - 1)$ -manifold with non-vanishing fundamental group, embedded rectilinearly in Euclidean n -space, R^n , and bounding there a domain with all its homotopy groups zero (including π_1). This leads to an example of an open set in R^n which is ULC^m , but whose boundary is not $1 - LC$, thus settling in the negative a question raised by Eilenberg and Wilder.¹

1. Let A and B be oriented circles with one common point, o , and let a space Z be formed by identifying the boundaries of two circular discs with the loops $A^{-3}(BA)^2$ and $B^{-5}(BA)^2$, respectively. More precisely, the boundary of disc 1 is divided into 7 equal arcs which are mapped onto A^{-1} , A^{-1} , A^{-1} , B , A , B , A , respectively, topologically save that both end-points of each arc are mapped onto o ; and similarly for disc 2. All boundary points of the discs are then identified with their images. This 2-dimensional space is familiar as an example of a polyhedron which is acyclic² but has $\pi_1 \neq 0$. That $\pi_1(Z) \neq 0$ is clear, since the only relations connecting its generators A and B are

$$(BA)^2 = A^3 = B^5, \quad (1)$$

which are among those satisfied by the generators of the icosahedral group; but $H^1(Z) = 0$, since the adjunction of $AB = BA$ to (1) leads to $A = B = 1$. Again, if C_1^2 and C_2^2 are 2-chains on simplicial subdivisions of the two discs, with boundaries A and B , respectively, any 2-cycle on Z is of the form $mC_1^2 + nC_2^2$. Its boundary, written additively, is $m(-A + 2B) + n(2A - 3B)$, and hence can only be zero identically in A and B if $m = n = 0$. There are therefore no non-zero 2-cycles, and $H^2(Z) = 0$.

2. If $n \geq 5$, some simplicial subdivision Σ_n^0 of the n -sphere S^n contains a simplicial subdivision K_0^2 of Z as a closed subcomplex.³ Let Σ_n^0 and K_0^2 be changed by a derivation⁴ into Σ_1^2 and K_1^2 , respectively. If then $K_1^2 = St(K_1^1)$ in Σ_1^2 , each simplex of $K_1^2 - K_1^1$ is uniquely expressible as the join $\sigma_a \sigma_b$, where $\sigma_a \in K_1^1$, $\sigma_b \in \Sigma_1^2 - K_1^1$. Hence each point x of $|K_1^2 - K_1^1|$ is uniquely expressible in the form $(1 - \tau)x_a + \tau x_b$, where $x_a \in |\sigma_a|$, $x_b \in |\sigma_b|$,

$\sigma_a\sigma_b$ being the carrier simplex of x , and $0 < \tau < 1$. (Cf. Lefschetz, *Algebraic Topology*, p. 292.) We denote the number τ by $\tau(x)$.

Let X be the closed set of points

$$|K_1^2| \cup \{x | \tau(x) \leq 1/2\}.$$

in $|K_1^2|$. It is evidently a polyhedron. We denote its boundary by M , and $S^n - X$ by Y . The set X is retractible, on itself, into $|K_1^2|$, by projection along the segments x_ax_a . Hence X and $X - M$ are acyclic; and therefore Y and $\text{Int } Y$ are acyclic, by the Alexander Duality Theorem. Any semilinear mapping ϕ_0 of S^1 into Y can be extended to a semilinear mapping ϕ_1 of E^2 into S^n which, since $n \geq 5$, can by arbitrarily small displacements of the vertices be made into a mapping ϕ_2 of E^2 into $S^n - |K_1^2|$. By projection of ϕ_2 outwards along the segments x_ax_b we obtain a mapping of E^2 into Y which is an extension of ϕ_0 . Hence $\pi_1(Y) = 0$. Similarly, $\pi_1(\text{Int } Y) = 0$.

However, $\pi_1(M) \neq 0$. Let ψ_0 be a semilinear and topological mapping of S^1 onto the circle A in $|K_1^2|$, covering the 1-simplexes $|\sigma_i^1|$ ($i = 1, 2, \dots, k$) in that order. Let $\sigma_i^1 = x_ix_{i+1}$, ($x_{k+1} = x_1$). For each i choose an n -simplex $\sigma_i^n = \sigma_i^1\sigma_i^{n-2}$ of K_1^n , such that $\sigma_i^{n-2} \in \Sigma_1^n - K_1^n$. Let ϵ be a positive number such that for all i , $U(x_i, \epsilon) \subseteq X$. For each i let $y_i z_i$ be a segment parallel to x_ix_{i+1} and similarly sensed, lying in the interior of $|\sigma_i^n|$ save for its end-points y_i, z_i , which are on the boundary and within ϵ of x_i and x_{i+1} , respectively. Join z_{i-1} to y_i by a path s_i in $(S^n - |K_1^2|) \cap U(x_i, \epsilon)$. The segments $y_i z_i$ and the paths s_i together determine a loop $\psi_1(S^1)$ in $X - |K_1^2|$, which is deformable into a mapping ψ_2 of S^1 into the circle A , by projecting points of $y_i z_i$ along segments x_ax_b (as above), and each point of s_i linearly onto x_i . This deformation is in X , and since $\psi_2(S^1)$ covers each σ_i^1 once positively, $\psi_2 \simeq \psi_0$ in A . Now ψ_0 is not null-homotopic in X , since this would imply $\psi_0 \simeq 0$ in $|K_1^2|$ by retraction, contrary to the fact. Hence ψ_2 and ψ_1 are not null-homotopic in X . Since ψ_1 is in $X - |K_1^2|$ it can be projected out along the segments x_ax_b to give a loop $\psi_3(S^1)$ in M which is not null-homotopic in X , nor *a fortiori* in M .

3. In a suitable subdivision of Σ_1^n the sets M , X and Y are the loci of subcomplexes M^{n-1} , X^n , Y^n ; and M^{n-1} is an $(n-1)$ -manifold. Indeed this is true if in the definitions of M , X and Y the sphere Σ_1^n is replaced by any once derived manifold M_1^n , and K_1^2 by any closed subcomplex K_1 . For each 1-simplex $|ab|$, with a in K_1 and b not, contains one point c of the set M . If the edges ab are "broken" one by one in any order, taking the points c as new vertices, then in the subdivided manifold M_2^n the rectilinear simplexes determined by the new vertices form a subcomplex M^{n-1} whose locus is precisely M ; and if X^n is $St(K_1)$ in M_2^n , $|X^n| = X$ and $|M_2^n - X^n| = Y$. Let c_0 be the new vertex on the edge a_0b_0 . By projection from

a_0 the part of M in each simplex $|a_0 b_0 \sigma|$ of M_1^n is mapped $(1, 1)$ onto $\text{Int } Y \cap |b_0 \sigma|$. We thus obtain a topological and semilinear mapping of $|St(c_0)$ in $M^{n-1}|$ onto a neighborhood of b_0 in the boundary of $|E^n|$, where $E^n = St(a_0 b_0)$ in M_1^n . Since M_1^n is a once-derived n -manifold, E^n is an n -element, and its boundary is a sphere. It follows that M^{n-1} is an $(n-1)$ -manifold.

4. Let $B(o, 1)$ stand for the "box" $|\xi_r| \leq 1$ in R^n with center o and side 2, and let $Y(o, 1)$ denote a semilinear homeomorph of some subdivision of Y , having one (closed) boundary $(n-1)$ -simplex $|\sigma_0^{n-1}|$ in $\xi_1 = -1$, and all other points in $\text{Int } B(o, 1)$. Let $B(a, \lambda)$ denote, for any point $a = (\alpha_r)$, and any positive λ , the box $|\xi_r - \alpha_r| \leq \lambda$, and let $Y(a, \lambda) = fY(o, 1)$, where f is the linear mapping that sends $B(o, 1)$ into $B(a, \lambda)$. Finally let e_i be the point $(2^{-i+2}, 1 + 2^{-i}, 0, 0, \dots, 0)$ of R^n , $Y_i = Y(e_i, 2^{-i})$ and

$$F = \bigcup_{i=1}^{\infty} Y_i \cup B(o, 1).$$

The set $\text{Int } F$ is ULC^∞ . This is equivalent to the assertion that $\text{Int } F$ is LC^∞ at all points of $\text{Fr } F$. If $e_\infty = (0, 1, 0, \dots, 0)$, then at all points except e_∞ , $\text{Fr } F$ has the character of an $(n-1)$ -manifold embedded rectilinearly in R^n . Therefore $\text{Int } F$ is certainly LC^∞ at all points of $\text{Fr } F$ except possibly e_∞ . The sets $B(o, 1)$ and Y_i are all acyclic, $Y_i \cap Y_j = 0$ for $i \neq j$, and $Y_i \cap B(o, 1)$ is an $(n-1)$ -simplex. Therefore the set

$$F_j = \bigcup_{i=1}^j Y_i \cup B(o, 1)$$

is acyclic for each j . Since any cycle on a compact subset of $\text{Int } F$ is necessarily contained in some F_j it follows that $\text{Int } F$ is acyclic. Similarly $\pi_1(Y_i) = \pi_1(B(o, 1)) = 0$, and therefore $\pi_1(F_j) = 0$. Since any mapping $f: S^1 \rightarrow \text{Int } F$ is necessarily into F_j for some j , $\pi_1(\text{Int } F) = 0$. By Hurewicz's theorem it follows that $\pi_r(\text{Int } F) = 0$ for $r \geq 1$.

Let g_j be the point $(0, 1 - 2^{-j}, 0, 0, \dots, 0)$ of R^n , and

$$F'_j = \bigcup_{i=j+1}^{\infty} Y_i \cup B(g_j, 2^{-j}).$$

Then F'_j is the homeomorph of F under a linear mapping of the whole of R^n onto itself. Hence $\text{Int } F'_j$ is the homeomorph of $\text{Int } F$, and therefore all its homotopy groups vanish. Moreover

$\text{Int } F \cap U_j = \text{Int } F \cap U(e_\infty, 2^{-j}) \subseteq \text{Int } F'_j \subseteq U(e_\infty, 2^{-j}) = \text{Int } F \cap U_{j-2}$. Hence every mapping of a sphere into $U_j \cap \text{Int } F$ is null-homotopic in $U_{j-2} \cap \text{Int } F$, i.e., $\text{Int } F$ is LC^∞ at e_∞ .

By Theorem 4 of Eilenberg and Wilder (*loc. cit.*) F is LC^∞ . However, $\text{Fr } F$ is not $1-LC$. For any fixed j let M_j be the boundary of Y_j , and

$f_0: S^1 \rightarrow M_j$ a semilinear mapping, not null-homotopic in M_j . By projection from a suitable point of the $(n-1)$ -simplex $|\sigma_j^{n-1}|$ in which Y_j meets $B(o, 1)$, f_0 can be deformed, on M_j , into a mapping g_0 in $M_j - |\sigma_j^{n-1}|$, and therefore in FrF . If g_0 were extendable to a mapping $g_1: E^2 \rightarrow FrF$, then $g_1|_{|\sigma_j^{n-1}|}(M_j)$ could be extended over $|\sigma_j^{n-1}|$ to give a mapping of E^2 into M_j . This would imply $f \simeq g_0 \simeq 0$, in M_j , contrary to hypothesis. Thus in every neighborhood of e_∞ there is a loop in FrF which is not null-homotopic in FrF : FrF is not $1 - LC$ at e_∞ .

On the other hand, it follows from a theorem of Wilder⁹ that since $IntF$ is ulc^∞ (in the sense of homology), FrF is lc^∞ .

¹ Eilenberg, S., and Wilder, R. L., "Uniform Local Connectedness and Contractibility," *Am. J. Math.*, **64**, 613-622, Problem 2 (1942).

² I.e., connected, and q -acyclic for $q \geq 1$. All homology groups are formed with integral coefficients.

³ Σ_0^2 , K_0^2 , etc., are abstract complexes. $|\sigma|$ denotes the (open) Euclidean simplex corresponding to the abstract simplex σ , and $|K| = \bigcup |\sigma|$ for $\sigma \in K$. If $K_1 \subseteq K_2$, StK_1 (in K_2) is the set of all simplexes with a vertex in K_1 .

It is in fact possible to embed K_0^2 as a subcomplex in S^4 , but the corresponding Y is not aspherical.

⁴ "Derived complex" = subdivision having one vertex for each simplex of the original complex, as in the barycentric division of the Euclidean complex.

⁵ $IntX$ = interior of X , FrX = frontier of $X = X - IntX$.

⁶ Breaking an edge ab in an abstract simplicial complex K is replacing each simplex $ab\sigma$ (the case $\sigma = 1$ included) by the two simplexes $ac\sigma$ and $cb\sigma$, where c is a new vertex, not in K .

⁷ Cf. Eilenberg and Wilder, *loc. cit.* A set $X \subseteq S$ is $q - LC$ at any point a of S (not necessarily in X) if given $\epsilon > 0$ there exists $\eta(\epsilon, a) > 0$ such that every mapping of S^n into $X \cap U(a, \eta)$ is extendable to a mapping of E^{q+1} into $X \cap U(a, \epsilon)$. " LC^q at a " = " $q - LC$ at a for $q \leq p$," " LC^∞ at a " = " $q - LC$ at a for all q ."

⁸ Vietoris, L., "Homologiegruppen der Vereinigung zweier Komplexe," *Monatshefte Math. Phys.*, **37**, 159-162 (1930). The analogous theorem for the fundamental group, Seifert, H., "Konstruktion 3-dimensionaler Räume," *Ber. Sächs. Akad.*, **83**, 26-66 (1931).

⁹ Wilder, R. L., "Generalized Closed Manifolds in n -Space," *Ann. Math.*, **35**, 876-903 (1934).

CYCLOTOMIC POWER CHARACTERS AND TRINOMIAL EQUATIONS IN A FINITE FIELD

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In another article¹ employing extensions of methods originally due to Kummer, we obtained relations concerning power characters of units in a cyclotomic field defined by θ , where θ is a primitive m th root of unity,

m being composite. Our investigations were not complete, however, as they did not apply to the case where m is prime. In the present paper we shall develop these methods much further and obtain a result (Theorem I) which may not only be applied to power characters but also trinomial equations in a finite field as well as to other associated topics.

We define

$$\Psi_{a,b}(\theta) = \sum_h \theta^{-bh + (a+b) \text{Ind}(\theta^h + 1)},$$

where g is a primitive root of the field $F(q')$ of order q' , θ is a primitive m th root of unity with $m = ln$ and l an odd prime, n any integer > 0 with $(l, n) = 1$, and h ranges over all values of the set $0, 1, 2, \dots, q' - 2$ except h' where $g^{h'} = -1$ in $F(q')$. If q is odd then $h' = (q' - 1)/2$, and if q' is even $h' = 0$. Also, $q' - 1 \equiv 0 \pmod{m}$. It is known² that

$$\Psi_{a,b}(\theta)\Psi_{a,b}(\theta^{-1}) = q', \quad (2)$$

if $a \not\equiv 0, b \not\equiv 0, a + b \not\equiv 0 \pmod{m}$; and

$$\Psi_{a,0}(\theta)\Psi_{0,b}(\theta) = -1 \quad (3)$$

$$\Psi_{0,0}(\theta) = q' - 2. \quad (3a)$$

In another paper³ we defined operations with polynomials, using exponents in a commutative ring R with a unity element and also defined what we called formal exponential differentiation of polynomials or quotients of polynomials of this kind and used these concepts to obtain certain congruences in an algebraic field.

For the particular application of this idea that we shall need here, it is sufficient to define the formal exponential derivative with respect to x of a polynomial,

$$f(x) = \alpha_n x^n + \alpha_{n-1} x^{n-1} + \dots + \alpha_0,$$

with the α 's in the ring R and x an indeterminate over R , as

$$\begin{aligned} \frac{\bar{d}f(x)}{dx} &= \bar{D}_x f(x) \\ &= n\alpha_n x^n + (n-1)\alpha_{n-1} x^{n-1} + \dots + \alpha_1 x, \end{aligned}$$

that is, we differentiate $f(x)$ in the ordinary way and then multiply by x . If A and B are two polynomials in x then we also define the formal exponential derivative of A/B as

$$\frac{B\bar{D}_x(A) - A\bar{D}_x(B)}{B^2}.$$

Suppose that $\theta = \alpha\beta$, where $\alpha = e^{2i\pi/l}$, $\beta = e^{2i\pi/n}$; and set

$$\Psi_{a,b}(x\beta) = \sum_h (x\beta)^{-bh + (a+b) \text{ ind } (g^h + 1)}$$

where, now, x is an indeterminate over the ring of algebraic integers in an algebraic field defined by β . From this we immediately obtain

$$\frac{1}{\Psi(x\beta)} \bar{D}\Psi(x\beta) = \sum_h \frac{-bh + (a+b) \text{ ind } (g^h + 1)(x\beta)^{-bh + (a+b) \text{ ind } (g^h + 1)}}{\Psi(x\beta)}, \quad (5)$$

where, since all our differentiation is with respect to x , we write \bar{D} for D , and since, in the main, a and b are fixed during our investigation we also write

$$\Psi_{a,b}(x\beta) = \Psi(x\beta).$$

Also, as (5) resembles the operation of finding the ordinary derivative of a logarithm, we write for brevity and suggestiveness,

$$\bar{D} \log \Psi(x\beta) = \frac{1}{\Psi(x\beta)} \bar{D}\Psi(x\beta).$$

It follows easily by induction that, as was stated in (10) of a previous paper³ (p. 25),

$$\bar{D}^{(n)}(AB) = \sum_{i=0}^n \binom{n}{i} \bar{D}^{(i)}(A) \bar{D}^{(n-i)}(B). \quad (6)$$

Applying this idea to (5) gives

$$\begin{aligned} \frac{\bar{d}^r \log \Psi(x\beta)}{dx^r} &= \frac{\bar{D}^r \Psi(x\beta)}{\Psi(x\beta)} + (r-1) \bar{D}^{r-1} \Psi(x\beta) \bar{D} \left(\frac{1}{\Psi(x\beta)} \right) + \\ &\quad \dots + \bar{D} \Psi(x\beta) \bar{D}^{r-1} \left(\frac{1}{\Psi(x\beta)} \right). \end{aligned}$$

The relation (2) may be written in the form

$$\Psi(\alpha\beta) \Psi(\alpha^{m-1} \beta^{m-1}) = q^l. \quad (8)$$

Now α is a primitive l th root of unity with l prime, and $(x^l - 1)/(x - 1)$ is irreducible in the field $K(\beta)$, since $(l, n) = 1$, consequently if x is an indeterminate, over $K(\beta)$,

$$\Psi(x\beta) \Psi(x^{m-1} \beta^{m-1}) = q^l + V(x) \frac{x^l - 1}{x - 1}, \quad (9)$$

where $V(x)$ is a polynomial in x with coefficients integers in the field $K(\beta)$.

We shall now prove the following lemma. If $c \geq 1$ and j is any integer ≥ 0 , and $s \not\equiv 0 \pmod{n}$, we have

$$\bar{D}^{(j)} \left[\sum_{i=0}^{cm-1} (x\beta^s)^i \right]_{x=1} \equiv (\text{mod } l). \quad (10)$$

For $s \equiv 0 \pmod{n}$ we have

$$\bar{D}^{(j)} \left[\sum_{i=0}^{cm-1} x^i \right]_{x=1} \equiv 0 \pmod{l}, \quad (10a)$$

for $j \not\equiv 0 \pmod{l-1}$. To prove this, we note that the left-hand member of (10) can be written as

$$\frac{d^j}{dx^j} \left[((x\beta^s)^{cm} - 1) \left(\frac{1}{x\beta^s - 1} \right) \right]. \quad (11)$$

since $\beta = e^{2\pi i/n}$ and $m = ln$, it follows that for $x = 1$, $(x\beta^s)^{cm} - 1 = 0$, and $d^k((x\beta^s)^{cm} - 1) = (cm)^k x^{cm} = (cln)^k \equiv 0 \pmod{l}$. Therefore the application of (6) to (11) gives for $x = 1$ every term $\equiv 0 \pmod{l}$, provided that $\bar{D}^{(n-i)} \left(\frac{1}{x\beta^s - 1} \right)$ has a denominator prime to l in the field $K(\beta)$, but each denominator will consist of some power of $\beta^s - 1$ with $s \not\equiv 0 \pmod{n}$. Now it is known⁴ that $\beta^s - 1$ will divide n and since $(l, n) = 1$ our proviso is satisfied and (10) is established. To prove (10a) we note that the left-hand member may be written in the form

$$\sum_{h=0}^{cm-1} h^j = \sum_{v,l} (vl + t)^j, \quad t = 0, 1, \dots, l-1; \quad v = 0, 1, \dots, cn-1.$$

Hence for $j \not\equiv 0$,

$$\sum_{h=0}^{cm-1} h^j \equiv cn \sum_{t=1}^{l-1} t^j \pmod{l}, \quad (11a)$$

and the second factor on the right is $\equiv 0 \pmod{l}$ if $j \not\equiv 0 \pmod{l-1}$. This completes the proof of the lemma.

From (9) we obtain,⁵ taking $i < l-1$,

$$\Psi(x^{m-1}\beta^{m-1}) = \frac{1 + lnv}{\Psi(x\beta)} + \frac{V(x\beta) x^l - 1}{\Psi(x\beta) x - 1}, \quad (12)$$

$$\bar{D}^{(j)}[\Psi(x^{m-1}\beta^{m-1})] = \bar{D}^{(j)} \left(\frac{1 + lnv}{\Psi(x\beta)} \right) + \bar{D}^{(j)} \left(\left(\frac{V(x)}{\Psi(x\beta)} \right) \left(\frac{x^l - 1}{x - 1} \right) \right). \quad (13)$$

Taking $c = 1$ in (11a) we note that every term in the expansion of the second term of the right-hand member of (13) is $\equiv 0 \pmod{l}$ for $x = 1$, since from (2), (3) and (3a), $\Psi(\beta)$ is prime to (l) . This gives for $i < l-1$,

$$[\bar{D}^{(j)}\Psi(x^{m-1}\beta^{m-1})]_{x=1} \equiv \bar{D}^{(j)} \left(\frac{1}{\Psi(x\beta)} \right) \pmod{l}, \quad (14)$$

where the right-hand member stands for

$$\bar{D}^{(i)} \left[\frac{1}{\Psi(x\beta)} \right]_{x=1}.$$

Also it may be shown that

$$[\bar{D}^{(i)} \Psi(x^{m-1} \beta^{m-1})]_{x=1} \equiv (-1)^i [\bar{D}^{(i)} \Psi(x \beta^{-1})]_{x=1} \pmod{l}, \quad (15)$$

by expanding both members of the equation and noting that for $x = 1$, $x^{m-1} = x$.

Application of (14) and (15) to (7) gives, modulo l , for $r < l$

$$[\bar{D}^{(r)} \log \Psi(x\beta)]_{x=1} \equiv \bar{D}^{(r)}(\Psi(\beta))\Psi(\beta^{-1}) + (r-1)(-1)\bar{D}^{(r-1)}\Psi(\beta) \cdot \bar{D}^{(1)}\Psi(\beta^{-1}) + \dots + (-1)^{r-1}\bar{D}^{(1)}\Psi(\beta)\bar{D}^{(r-1)}\Psi(\beta^{-1}), \quad (16)$$

where the symbol $\bar{D}^{(i)}(\Psi(\beta))$ stands for

$$[\bar{D}_x^{(i)}(\Psi(x\beta))]_{x=1}.$$

Set

$$-bh + (a+b) \text{ ind } (g^h + 1) = C_h \quad (17)$$

and

$$-bk + (a+b) \text{ ind } (g^k + 1) = C_k, \quad (18)$$

by which, together with (16) and the definition (1), we obtain

$$[\bar{D}^{(r)} \log \Psi(x\beta)]_{x=1} = \sum_h \sum_k (C_h' C_k' - (r-1) C_h^{r-1} C_k + \dots) (\beta)^{ch - ck} \pmod{l}. \quad (19)$$

or

$$[\bar{D}^{(r)} \log \Psi(x\beta)]_{x=1} \equiv \sum_h \sum_k C_h (C_h - C_k)^{r-1} \beta^{ch - ck} \pmod{l}, \quad (20)$$

h and k ranging independently over the values $0, 1, 2, \dots, q^t - 2$, excepting $(q^t - 1)/2$ if q is odd and zero if $q = 2$. Put

$$g^{k-h} = g^{h'}; \quad \frac{g^k + 1}{g^h + 1} = g^{k'}.$$

Then the right-hand member of (20) may be written

$$[-b \text{ ind } (g^{h'} - 1) + (a+b) \text{ ind } (g^{k'} - 1) - a \text{ ind } (g^{h'} - g^{h'})] \times (bk' - (a+b)h')^{r-1} \beta^{bk' - (a+b)h'}, \quad (21)$$

where h' and k' each range over $1, 2, \dots, q^t - 2$ but $h' \neq k'$. We now break (21) down into three parts

$$\sum_h \sum_k -\beta^{bk - (a+b)h} (bk - (a+b)h)^{r-1} \text{ ind } (g^h - 1) \quad (I)$$

$$\sum_h \sum_k (a+b) \beta^{bk-(a+b)h} (bk - (a+b)h)^{r-1} \text{ind } (g^k - 1) \quad (\text{II})$$

$$- \sum_h \sum_k a \beta^{bk-(a+b)h} (bk - (a+b)h)^{r-1} \text{ind } (g^k - g^h). \quad (\text{III})$$

where we now re-employ the symbols h and k to range independently over $1, 2, \dots, q^t - 2$, but $h \neq k$. We see that (I) may be written as

$$b \sum_h \beta^{-ah} (-ah)^{r-1} \text{ind } (g^h - 1) - b \sum_h \sum_k \beta^{bk-(a+b)h} (bk - (a+b)h)^{r-1} \text{ind } (g^h - 1), \quad (22)$$

where now h and k each range independently over all values from 1 to $q^t - 2$, inclusive, without exception. If $b \not\equiv 0 \pmod{n}$, then for each fixed value of h , the second term of (22) can be expanded, and when we carry out the summation with respect to k then each term of that expansion will be divisible by l (except the last one), by the lemma (10), (which could have been stated in the form $\sum_{h=0}^{q^t-2} h^i \beta^{nh} \equiv 0 \pmod{l}$ for $\beta^n \neq 1$ and $0^0 = 1$), and (22) reduces to

$$(-a)^{r-1} b \sum_h \beta^{-ah} h^{r-1} \text{ind } (g^h - 1) + b \sum_h \beta^{-(a+b)h} (-(a+b)h)^{r-1} \text{ind } (g^h - 1). \quad (\text{IV})$$

If $a+b \not\equiv 0 \pmod{n}$, then, in a similar fashion, (II) can be reduced to

$$-(-a)^{r-1} (a+b) \sum_k \beta^{-ak} k^{r-1} \text{ind } (g^k - 1) - \sum_k (bk)^{r-1} (a+b) \beta^{bk} \text{ind } (g^k - 1). \quad (\text{V})$$

To reduce (III) introduce in it the terms corresponding to $h = 0$ and $k = 0$ with $h = k$, to offset which we add

$$a(-(a+b))^{r-1} \sum_h \beta^{-(a+b)h} h^{r-1} \text{ind } (g^h - 1) + ab^{r-1} \sum_h \beta^{bh} h^{r-1} \text{ind } (g^h - 1). \quad (\text{VI})$$

Then in the resulting expression in (III) we set $h_1 + k_1$ for k and h_1 for h , and this gives

$$-a \sum_{h_1} \sum_{k_1} (h_1 + \text{ind } (g^{k_1} - 1)) (bk_1 - ah_1)^{r-1} \beta^{bk_1 - ah_1} \quad (\text{VIa})$$

$$h_1 = 0, 1, 2, \dots, q^t - 2; \quad k_1 = 1, 2, \dots, q^t - 2,$$

or

$$-a \sum_{h_1} \sum_{k_1} \beta^{bk_1 - ah_1} h_1 (bk_1 - ah_1)^{r-1} - a \sum_{h_1} \sum_{k_1} \beta^{bk_1 - ah_1} (bk_1 - ah_1)^{r-1} \text{ind } (g^{k_1} - 1).$$

By (10) this reduces to zero modulo l , if $a \not\equiv 0 \pmod{n}$. Hence we add

the quantities given in (IV), (V), and (VI) which were the terms of the right-hand side of (20) and obtain for $r < l$,

$$\left[\frac{\bar{d}^{r-1}}{dx^{r-1}} \left(\frac{\bar{D}\Psi(x\beta)}{\Psi(x\beta)} \right) \right]_{x=1} = (-1)^r a^r \sum_k \beta^{-ak} A(k) + (-1)^{r+1} (a+b)^r \sum_k \beta^{-(a+b)k} A(k) - b^r \sum_k \beta^{bk} A(k) \pmod{l}, \quad (23)$$

where k ranges over the values $1, 2, \dots, q^l - 2$, and $A(k) = k^{r-1} \pmod{q^l - 1}$. This gives

THEOREM I. *If a and b are integers such that $a \not\equiv 0 \pmod{n}$, $b \not\equiv 0 \pmod{n}$, $a+b \not\equiv 0 \pmod{n}$, $\beta = e^{2\pi i/n}$, $\alpha = e^{2\pi i/l}$, $ln = m$; $(n, l) = 1$; l an odd prime and*

$$\Psi(x\beta) = \sum_h (x\beta)^{-bh + (a+b) \text{ ind } (g^h+1)},$$

where h ranges over the integers $0, 1, \dots, q^l - 2$, excepting $(q^l - 1)/2$; q is an odd prime, g is a primitive root in the finite field of order q^l ; with $q^l \equiv 1 \pmod{m}$. Then the congruence (23) holds where $r < l$, and the \bar{d} symbols on the left indicate exponential differentiation as defined in the first part of the present article.

We now consider other conditions related to (23). If $r = l - 1$ and $n \neq 1$ then in reducing (I), (II), and (III) we find, using (10) and (10a), that we obtain (V) and (VI) without the restrictions $b \not\equiv 0 \pmod{n}$ and $a+b \not\equiv 0 \pmod{n}$. To obtain (VIb) we encounter, however, the term $a^{l-2} \sum_h h_1^{l-1} \beta^{-bh}$ in the development and this is not $\equiv 0 \pmod{l}$ when $a \equiv 0 \pmod{n}$. Hence, we have the result that (23) holds for $a \not\equiv 0 \pmod{n}$, $b \not\equiv 0 \pmod{m}$, $a+b \not\equiv 0 \pmod{m}$. If $n = 1$, then for $r = l - 1$ we have $\beta = 1$ and

$$\sum_{h_1} h_1^r = \sum_{h_1} h_1^r \beta^{-ah} \not\equiv 0 \pmod{l},$$

but we see, however, because of (10a) that (23) holds for $a \not\equiv 0 \pmod{m}$, $b \not\equiv 0 \pmod{m}$, $a+b \not\equiv 0 \pmod{m}$, if $r < l - 1$. These results enable us to apply (23) to obtain the expressions found for l th power character $((1 - \alpha\beta)/p)$, in a previous paper,¹ where p is a prime ideal in $K(\theta)$. For $n = 1$, $r < l - 1$ we derive from (23) a result which may be written as

$$\left[\frac{d^r \log \Psi(e^*)}{dv^r} \right]_{v=0} = ((-1)^r a^r + (-1)^{r-1} (a+b)^r - b^r) \sum_k A(k),$$

and from this we easily obtain a relation due originally to Kummer² involving the power character (ϵ/q) where $\epsilon = (1 - \alpha')/(1 - \alpha)$ and q is a prime ideal in $K(\alpha)$. Applications to trinomial equations in a finite

field as well as other applications to power characters will be given elsewhere.

¹ *Am. J. Math.*, **47**, 140-147 (1925).

² Mitchell, H. H., *Trans. Am. Math. Soc.*, **17**, 167 (1916).

³ Vandiver, H. S., these PROCEEDINGS, **28**, 25 (1942).

⁴ Hilbert, D., *Gesammelte Abhandlungen*, Bd. I, Berlin, Springer (1932), pp. 199, 203.

⁵ The derivations of the formulas (12)-(20) which follow resemble some of the steps in our previous paper,¹ but from then on the treatment is a bit different.

⁶ Vandiver, H. S., *Ann. Math.*, **30**, 487 (1929), relation (1).

THE STABILITY OF DIFFERENTIAL EQUATIONS WITH PERIODIC COEFFICIENTS

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The following theorem will be proved:

If $f(t)$ is a real, continuous, non-constant function of period π which is such that

$$n^2 \leq f(t) \leq (n+1)^2, \quad (-\infty < t < \infty), \quad (1)$$

for some integer n , then the characteristic exponents of

$$x'' + f(t)x = 0 \quad (2)$$

are distinct and of stable type.

A weakened form of this theorem, a form in which the signs of equality are excluded in (1) for every t , must be known for a long time (see, for example, Borg,¹ where reference is made to a lecture of Breuling). Actually, the truth of the theorem, at least of the weaker wording, is indicated by the diagrams available for such cases as the equations of Mathieu and Meissner; cf., e.g., Strutt.² However, I could not find a proof for even the weaker form of the theorem in the literature.

Whether or not the periodic function $f(t)$ satisfies (1), there exist a number $\rho \neq 0$ and a solution $x = x(t) \neq 0$ of (2) such that

$$x(t + \pi) = \rho x(t), \quad (3)$$

where ρ and $x(t)$ can be complex-valued. The number ρ is a root of the characteristic equation of (2), a reciprocal quadratic equation with real coefficients. Hence, if ρ is not real, its absolute value is 1. If the trivial case where $f(t)$ is a constant is excluded, the assertion to be proved is that ρ is not real in virtue of (1).

Suppose, if possible, that ρ is real. Then if $\phi(t)$ is defined, in terms of the solution $x(t)$ occurring in (3), by

$$x(t) = |\rho|^{t/\pi} \phi(t),$$

it is seen that

$$\phi(t + \pi) = \epsilon \phi(t), \text{ where } \epsilon = \operatorname{sgn} \rho = \pm 1.$$

Hence $\phi(t)$ has 2π as a period. Since $f(t)$ is real, the real and imaginary parts of $x(t)$ are solutions of (2). Consequently, (2) possesses a non-trivial solution of the form

$$x(t) = |\rho|^{t/\pi} \psi(t) \not\equiv 0, \quad (4)$$

where $\psi(t)$ is real and of period 2π .

Since the solution (4) is real, it follows from the inequalities (1), from Sturm's comparison theorem, and from the assumption that $f(t)$ is not constant, that

$$2n < N < 2(n + 1), \quad (5)$$

where N denotes the number of zeros of the solution (4) on a half-open interval of length 2π , say on $0 \leq t < 2\pi$.

On the other hand, (4) shows that $x(t)$ and $\psi(t)$ have the same zeros. Since $\psi(t)$ is periodic, the number N of zeros of $\psi(t)$ on a period, $0 \leq t < 2\pi$, is even. Since this contradicts (5), the proof is complete.

¹ Borg, G., *Ark. f. Matemat., Astr. o. Fysik*, 31, No. 1, p. 28.

² Strutt, M. J. O., *Lamésche, Mathieusche und Verwandte Funktionen in Physik und Technik*, Berlin, 1932, pp. 24 and 40.

ON SOME EXPONENTIAL SUMS

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It seems to have been known for some time¹ that there is a connection between various types of exponential sums, occurring in number-theory, and the so-called Riemann hypothesis in function-fields. However, as I was unable to find in the literature a precise statement for this relationship, I shall indicate it here, and derive from it precise estimates for such sums, including the Kloosterman sums.

Let k be a finite field of q elements; consider the field $k(t)$ of rational functions in one transcendental element t , with coefficients in k ; geo-

metrically, this is the function-field, over the ground-field k , of a projective straight line. On that straight line, we consider divisors, i.e., formal sums of points with integral (positive or negative) coefficients; and we limit ourselves, once for all, to divisors which are rational over k , i.e., such that conjugate points over k have the same coefficient. A divisor is called finite if it does not contain the point at infinity with a non-zero coefficient. Except for the notation, finite positive divisors are essentially the same as ideals in the ring $k[t]$; to every such divisor a , we attach the polynomial $P_a(t) = t^n + a_1 t^{n-1} + \dots + a_n$ which generates the corresponding ideal, i.e., whose zeros are the points in a , with multiplicities respectively equal to their coefficients in a ; as a is assumed to be rational over k , $P_a(t)$ has its coefficients in k ; and n is the degree of a . Every finite divisor m can be written as $m = a - b$, where a, b are finite positive divisors; to m , we attach the function $R_m(t) = P_a(t)/P_b(t)$; we have $m \sim 0$ if and only if a and b , i.e., $P_a(t)$ and $P_b(t)$, are of the same degree, and then there is one and only one function in $k(t)$ having m as its divisor and taking the value 1 at infinity, viz., $R_m(t)$ itself.

Let χ be a character of the multiplicative group k^* of the non-zero elements in k . Let b be a finite divisor, consisting of the points ξ_v with the coefficients a_v ; if $R(t)$ is in $k(t)$, we shall write $R(b) = \prod_v R(\xi_v)^{a_v}$ whenever none of the $R(\xi_v)$ is 0 or ∞ ; as b is rational over k , $R(b)$ is in k . We shall assume that no a_v is a multiple of the order of χ .

Furthermore, let ω be a character of the multiplicative group of power series in an indeterminate T with coefficients in k ; we assume that ω has the value 1 for every series reduced to a monomial cT^m . According to the usual definition, we say that ω has the conductor (T^N) if it has the value 1 for every power-series which is $\equiv 1 \pmod{T^N}$, and if N is the smallest integer with that property. Then the values of ω are p^s -th roots of unity, if p is the characteristic of k , and s is such that $p^s \geq N$. We shall write, for $x \in k$, $\lambda(x) = \omega(1 - xT)$. To every function $R(t)$ in $k(t)$, we can attach a power-series $R(1/T)$, arising from the expansion of the rational function $R(1/T)$ according to increasing powers of T ; this is no other than the usual expansion of $R(t)$ at infinity. Then $\omega[R(1/T)]$ is defined; in particular, as $\omega(T) = 1$, we have $\omega[(1 - xT)/T] = \lambda(x)$. Now, for every finite divisor m with no point in common with b , we write

$$\varphi(m) = \omega[R_m(1/T)] \cdot \chi[R_m(b)]. \quad (1)$$

This depends multiplicatively upon m , i.e., $\varphi(m + n) = \varphi(m)\varphi(n)$. Furthermore, if $m \sim 0$, and if there is a function $R(t)$ in $k(t)$, having m as its divisor, taking the value 1 at every point ξ_v , and such that $R(1/T) \equiv 1 \pmod{T^N}$, we have $\varphi(m) = 1$; for $R(t)$ can then be no other than $R_m(t)$. According to well-known definitions, this shows that $\varphi(m)$ is an Abelian character over the field $k(t)$, whose conductor consists of the point at

infinity with the coefficient N , and of the points ξ_v with the coefficient 1; if d is the number of points ξ_v in \mathfrak{b} , the degree of that conductor is therefore $N + d$; hence, by a known theorem,² the L -series belonging to this character is a polynomial of degree $N + d - 2$; calling α_i its roots, we have thus

$$\sum_a \varphi(a) \cdot u^{n(a)} = \prod_{i=1}^{N+d-2} (1 - \alpha_i u), \quad (2)$$

where the sum in the left-hand side is extended to all finite positive divisors a with no point in common with \mathfrak{b} , and where $n(a)$ is the degree of a . Writing that the terms in u are equal on both sides, we get

$$\sum \varphi(a) = -\sum_i \alpha_i, \quad (3)$$

where the sum in the left-hand side is now extended only to the finite positive divisors of degree 1. These are in one-to-one correspondence with the polynomials $P_a(t) = t - x$, with $x \in k$. For such a divisor, we have

$$R_a(1/T) = P_a(1/T) = (1 - xT)/T,$$

hence $\omega[R_a(1/T)] = \lambda(x)$, and also

$$R_a(\mathfrak{b}) = \prod_v (\xi_v - x)^{a_v} = (-1)^a R_b(x),$$

with $a = \sum a_v$. Then (3) can be written as

$$\sum \lambda(x) \chi[R_b(x)] = (-1)^{a+1} \sum \alpha_i, \quad (4)$$

where the sum in the left-hand side is over all the elements x of k , other than the ξ_v , if any of these is in k . We may extend that sum to all elements x of k by agreeing that $\chi(0) = \chi(\infty) = 0$.

By class-field theory, the character $\varphi(\mathfrak{m})$ belongs to an Abelian extension of $k(t)$, and its L -series divides the zeta-function of that extension. Therefore, by the Riemann hypothesis,³ all the α_i have the absolute value \sqrt{q} , hence

$$|\sum \lambda(x) \chi[R_b(x)]| \leq (N + d - 2) \sqrt{q}. \quad (5)$$

For instance, we can define a character ω , of conductor (T^2) , by putting, for every series of constant term 1:

$$\omega(1 + x_1 T + x_2 T^2 + \dots) = -\psi(x_1),$$

where ψ is a character of the additive group of k , not everywhere equal to 1. This gives

$$|\sum \psi(x) \chi[R_b(x)]| \leq d \sqrt{q}.$$

If the characteristic p of k is not 2, we have $d = 2$ for $R_b(t) = t^2 - a$, $a \neq 0$; hence, in that case,

$$|\sum \psi(x) \chi(x^2 - a)| \leq 2 \sqrt{q}.$$

If, in this, we take for χ the character of k^* of order 2 (equal to 1 for squares, and to -1 for non-squares, in k^*), an elementary transformation⁴ shows that the sum in the left-hand side is identical with the so-called Kloosterman sum $\sum \psi(cx + dx^{-1})$, for $4cd = a$; hence

$$|\sum_{x \neq 0} \psi(cx + dx^{-1})| \leq 2 \sqrt{q},$$

and, in the case of a prime field of p elements, with $p \neq 2$:

$$|\sum_{x=1}^{p-1} e^{2\pi i/p} (cx + d/x)| \leq 2 \sqrt{p}.$$

Furthermore, it is easily seen, e.g., by induction on n , that, if $F(x)$ is¹ any polynomial in x of degree n , with coefficients in k , such that $F(0) = 0$, there exists at least one character ω , of conductor (T^N) for some $N \leq n + 1$, such that, with the above notations, $\lambda(x) = \psi[F(x)]$. Then (5) gives:

$$|\sum \psi[F(x)] \chi[R_b(x)]| \leq (n + d - 1) \sqrt{q}.$$

¹ Cf., for example, H. Rademacher's excellent report on analytic number theory, *Bull. A. M. S.*, **48**, 379-401 (1942).

² Weissinger, J., *Hamb. Abhandl.*, **12**, 115-126 (1938).

³ Weil, A., *Pub. Inst. Math. Strasbourg (N.S., no. 2)*, pp. 1-85 (1948).

⁴ Davenport, H., *Crelles J.*, **169**, 158-176 (1933); cf. in particular Th. 5, p. 172.

ON SPACES WITH VANISHING LOW-DIMENSIONAL HOMOTOPY GROUPS

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This note contains an investigation of the relationships between some of the homotopy and homology groups of an $(n - 1)$ -connected space (i.e., a pathwise connected topological space whose homotopy groups of dimensions $< n$ all vanish).

Let X be an $(n - 1)$ -connected space, and let A be a set of generators for the n th homotopy group, $\pi_n(X)$. For each $\alpha \in A$, let E_α^{n+1} be an $(n + 1)$ -cell with boundary S_α^n ; let y_α be a fixed reference point of S_α^n ; x_0 a fixed reference point in X ; and let $f_\alpha: (S_\alpha^n, y_\alpha) \rightarrow (X, x_0)$ be a mapping representing the element $\alpha \in \pi_n(X)$. Suppose that $\bigcup_{\alpha \in A} E_\alpha^{n+1}$ is topologized so that the cells E_α^{n+1} are mutually separated and let E be the topological space obtained from $\bigcup_{\alpha \in A} E_\alpha^{n+1}$ by identifying all the points y_α to a single

point y_0 . Let S be the subset of E obtained from $\bigcup_{\alpha \in A} S_\alpha^n$ by the above identification. Since the mappings f_α carry y_α into x_0 , they induce a mapping $f: (S, y_0) \rightarrow (X, x_0)$. Finally let X^* be the identification space obtained from $E \cup X$ by identifying each point $y \in S$ with its image $f(y) \in X$. Then X may, in an obvious way, be considered as a subspace of X^* and the above identification induces a map $F: (E, S) \rightarrow (X^*, X)$ such that $F|S = f$. It is easy to see that X^* is n -connected. The results of this note are based on a consideration of the relations between the homology and homotopy groups of X^* and X .

The map $F: (E, S) \rightarrow (X^*, X)$ induces homomorphisms $F_k: \pi_{n+k}(E, S) \rightarrow \pi_{n+k}(X^*, X)$. The identity map $p^\alpha: (E_\alpha^{n+1}, S_\alpha^n) \rightarrow (E, S)$ induces isomorphisms $p_k^\alpha: \pi_{n+k}(E_\alpha^{n+1}, S_\alpha^n) \rightarrow \pi_{n+k}(E, S)$.

We shall make use of the following unpublished results of J. H. C. Whitehead:

If $k < n - 1$, then (1) $\pi_{n+k}(E, S)$ is the weak direct sum of the subgroups $p_k^\alpha(\pi_{n+k}(E_\alpha^{n+1}, S_\alpha^n))$. (2) F_k is an isomorphism onto. Hence for $k < n - 1$, $\pi_{n+k}(X^*, X) \approx \sum_{\alpha \in A} \pi_{n+k}(E_\alpha^{n+1}, S_\alpha^n) \approx \sum_{\alpha \in A} \pi_{n+k-1}(S_\alpha^n)$.

The group $\pi_{n+k+1}(E^{n+k}, S^{n+k-1})$ is isomorphic under the boundary operator with $\pi_{n+k}(S^{n+k-1})$; it is cyclic of order two if $n + k > 3$. Let $h_k: (E^{n+k+1}, S^{n+k}) \rightarrow (E^{n+k}, S^{n+k-1})$ represent a generator of this group, and let $h_k^0 = h_k|S^{n+k}$. By the Freudenthal "suspension" theorems we may assume that h_{k+1}^0 is the suspension of h_k^0 .

If $n + k > 3$, superposition with h_k induces a homomorphism $\tilde{\eta}_k: \pi_{n+k}(X^*, X) \rightarrow \pi_{n+k+1}(X^*, X)$; if $\alpha \in \pi_{n+k}(X, X)$ is represented by a mapping $g: (E^{n+k}, S^{n+k-1}) \rightarrow (X^*, X)$, then $\tilde{\eta}_k(\alpha)$ is represented by $gh_k: (E^{n+k+1}, S^{n+k}) \rightarrow (X^*, X)$. Similarly, superposition with h_{k+1}^0 induces homomorphisms

$$\begin{aligned}\eta_k: \pi_{n+k}(X) &\rightarrow \pi_{n+k+1}(X) \\ \eta_k^*: \pi_{n+k}(X^*) &\rightarrow \pi_{n+k+1}(X^*).\end{aligned}$$

The homomorphisms η_k , η_k^* , $\tilde{\eta}_k$ combine with the homomorphisms of the homotopy and homology sequences of (X^*, X) and the natural homomorphisms of the homotopy sequence into the homology sequence to produce a double ladder:

$$\begin{array}{ccccccc} \partial_2 & & i_1 & & j_1 & & \partial_1 \\ \dots \rightarrow \pi_{n+1}(X) & \xrightarrow{i_1} & \pi_{n+1}(X^*) & \xrightarrow{j_1} & \pi_{n+1}(X^*, X) & \xrightarrow{\partial_1} & \pi_n(X) \\ \\ \partial_3 & \downarrow \eta_1 & i_2 & \downarrow \eta_1^* & j_2 & \downarrow \tilde{\eta}_1 & \partial_2 & \downarrow \eta_0 & i_1 & & j_1 \\ \dots \rightarrow \pi_{n+2}(X) & \rightarrow \pi_{n+2}(X^*) & \rightarrow \pi_{n+2}(X^*, X) & \rightarrow \pi_{n+1}(X) & \rightarrow \pi_{n+1}(X^*) & \rightarrow & & & & & \\ & & & & & & \partial_1 & & & & \\ & & & & & & \pi_{n+1}(X^*, X) & \rightarrow \pi_n(X) & & & \end{array}$$

$$\begin{array}{ccccccccccc}
 \partial'_3 & \downarrow \rho_2 & i'_2 & \downarrow \rho_2^* & j'_2 & \downarrow \bar{\rho}_2 & \partial'_2 & \downarrow \rho_1 & i'_1 & \downarrow \rho_1^* & j'_1 \\
 \dots \rightarrow & H_{n+2}(X) \rightarrow & H_{n+2}(X^*) \rightarrow & H_{n+2}(X^*, X) \rightarrow & H_{n+1}(X) \rightarrow & H_{n+1}(X^*) \rightarrow & & & & & \\
 & & & & & & \downarrow \bar{\rho}_1 & \partial'_1 & \downarrow \rho_0 & & \\
 & & & & & & H_{n+1}(X^*, X) \rightarrow & H_n(X) & & &
 \end{array}$$

The homomorphisms denoted by ρ are the natural homomorphisms of the homotopy groups into the homology groups; the homomorphisms i_k and i'_k are those induced by the identity map $i: X \rightarrow X^*$; the homomorphisms j_k and j'_k are those induced by the identity map $j: (X^*, \phi) \rightarrow (X^*, X)$; the homomorphisms ∂_k and ∂'_k are those induced by the homotopy and homology boundary operators. The rows of the diagram are the homotopy and homology sequences of the pair (X^*, X) ; they are each *exact*² in the sense that the kernel of each homomorphism is the image of the preceding one. The homomorphisms of the diagram satisfy commutativity relations which state that for each square in the diagram, the two homomorphisms of the group in the upper left hand corner of the square into the group in the lower right hand corner are the same.

In the arguments to follow use is made of the Hurewicz theorems³ that the homomorphisms ρ_0 , $\bar{\rho}_1$ and ρ_1^* are isomorphisms onto. Examination of the diagram then yields the following results: (1) If $n > 1$, ρ_1 is onto. This result was proved by Fox² and independently by Hopf⁴ for the case where X is a complex. (2) If $n > 2$, the kernel of ρ_1 is the image of η_0 . Hence we have:

THEOREM 1. *If $n > 2$, $H_{n+1}(X) \approx \pi_{n+1}(X)/\text{Image } \eta_0$.*

Suppose now that $n > 3$. Then $\bar{\eta}_1$ is onto and the kernel of $\bar{\eta}_1$ is $2\pi_{n+1}(X^*, X)$. Moreover, since $\pi_{n+1}(S^n)$ is cyclic of order two, η_0 maps $2\pi_n(X)$ into zero. Define a homomorphism $\varphi: \pi_{n+2}(X^*) \rightarrow \pi_n(X)/2\pi_n(X)$ as follows: if $\alpha \in \pi_{n+2}(X^*)$, choose $\beta \in \pi_{n+1}(X^*, X)$ such that $\bar{\eta}_1(\beta) = j_2(\alpha)$ and let $\varphi(\alpha)$ be the coset of $2\pi_n(X)$ which contains $\partial_1(\beta)$. Then the image of φ is $\text{Kernel } \eta_0/2\pi_n(X)$ and the kernel of φ is the subgroup Q of $\pi_{n+2}(X^*)$ generated by the subgroups $\text{Kernel } j_2$ and $\text{Image } \eta_1^*$. Since the homotopy sequence is exact, $\text{Kernel } j_2 = \text{Image } i_2$; and since X^* is n -connected and $n+1 > 2$, $\text{Image } \eta_1^* = \text{Kernel } \rho_2^*$. It follows easily that $\pi_{n+2}(X^*)/Q \approx H_{n+2}(X)/\text{Image } \rho_2$.

The group $\text{Image } \rho_2$ is the group $\sum_{n+2}(X)$ of spherical homology classes. Hence we have:

THEOREM 2. *If $n > 3$, $H_{n+2}(X)/\sum_{n+2}(X) \approx \text{Kernel } \eta_0/2\pi_n(X)$.*

Let M^{n+2} be the join of the complex projective plane M^4 with an $(n-3)$ -sphere. The $(n+2)$ -nd integral homology group of M^{n+2} is infinite cyclic; let z be one of its generators. It then follows easily that

COROLLARY. *If $n > 2$ and $w \in H_{n+2}(X)$, there is a map $f: M^{n+2} \rightarrow X$ such that $f_*(z) = w$.*

If we further assume that $\pi_{n+2}(X) = 0$, further results can be obtained.

In this case $H_{n+1}(X)/\sum_{n+1}(X)$ is isomorphic with $H_{n+1}(X^*)/\sum_{n+1}(X^*)$. The latter group can be computed by applying Theorem 2 to the space X^* and we have⁵

THEOREM 3. If $n > 3$ and $\pi_{n+2}(X) = 0$, then

$$H_{n+1}(X)/\sum_{n+1}(X) \approx H_{n+1}(X)/2H_{n+1}(X) + {}_2(\pi_n(X)),$$

where ${}_2(\pi_n(X))$ is the subgroup of elements of $\pi_n(X)$ of order 2.

Summarizing, we see that if X is an $(n-1)$ -connected space and $n > 3$, the groups $H_{n+1}(X)$ and $H_{n+2}(X)/\sum_{n+2}(X)$ are determined by the homotopy groups $\pi_n(X)$ and $\pi_{n+1}(X)$ and the homomorphism $\eta_0: \pi_n(X) \rightarrow \pi_{n+1}(X)$. If also $\pi_{n+2}(X) = 0$, then $H_{n+2}(X)$ and $H_{n+3}(X)/\sum_{n+3}(X)$ are determined by $\pi_n(X)$, $\pi_{n+1}(X)$, and η_0 .

The above results are closely connected with recent work of Eilenberg and MacLane.⁶ These authors have constructed for each abelian group π and each integer n an abstract complex $K(\pi, n)$ with the property that if X is an $(n-1)$ -connected topological space such that $\pi_i(X) = 0$ for $n < i < n+h$, then the homology groups of dimension $< n+h$ of X and $K(\pi_n(X), n)$ are isomorphic, while the $(n+h)$ th homology group of $K(\pi_n(X), n)$ is isomorphic with $H_{n+h}(X)/\sum_{n+h}(X)$. The k th homology group of $K(\pi, n)$ is denoted by $H_k(\pi, n)$, and the k th cohomology group of $K(\pi, n)$ with coefficients in an abelian group G is denoted by $H^k(\pi, n; G)$. The results above enable us to calculate explicitly some of the groups $H_k(\pi, n)$ with the following results:

THEOREM 4. If $n > 1$, $H_{n+1}(\pi, n) = 0$. If $n > 3$, $H_{n+2}(\pi, n) \approx \pi/2\pi$. If $n > 3$, $H_{n+3}(\pi, n) \approx 2\pi$.

In recent unpublished work,⁷ Eilenberg and MacLane have defined for each space X such that $\pi_i(X) = 0$ for $i < n$ and $n < i < n+h$ a cohomology class k^{n+h+1} of $K(\pi_n(X), n)$ with coefficients in $\pi_{n+1}(X)$ and studied the influence of k^{n+h+1} on the homology groups of X . In the case $h = 1$ and $n > 2$, the cohomology class k^{n+2} will be described below.

Application of the universal coefficient theorem⁸ shows that for any abelian group G the group $H^{n+2}(\pi, n; G)$ is naturally isomorphic with the group of homomorphisms of $H_{n+2}(\pi, n)$ into G . Call this isomorphism λ . Now if $n > 2$, η_0 is a homomorphism of $\pi_n(X)$ into $\pi_{n+1}(X)$ which maps $2\pi_n(X)$ into zero. Hence η_0 induces a homomorphism $\eta: \pi_n(X)/2\pi_n(X) \rightarrow \pi_{n+1}(X)$. Let μ be the isomorphism described above; $\mu: H_{n+2}(\pi_n(X), n) \approx \pi_n(X)/2\pi_n(X)$. Then $\eta\mu$ is a homomorphism of $H_{n+2}(\pi_n(X), n)$ into $\pi_{n+1}(X)$ and

THEOREM 5. $\lambda(k^{n+2}) = \eta\mu$.

The abstract complex $K(\pi, n)$ has enough simplicial structure that the \cup, \cap products defined by Steenrod⁹ for simplicial complexes can be introduced. As in Steenrod's paper, these products are used to define a homomorphism $Sg_{n-2}: H^n(\pi, n; G) \rightarrow H^{n+2}(\pi, n; G')$ whenever a commutative self-

pairing of G to G' is defined and the products $g_1 \cdot g_2$ ($g_1, g_2 \in G$) all have order two.

The group $H^n(\pi, n; G)$ is isomorphic with the group of homomorphisms of $H_n(\pi, n)$ into G , and $H_n(\pi, n)$ is isomorphic with π . Hence $H^n(\pi, n; \pi)$ is isomorphic with the group of endomorphisms of π ; let d^n be the element of $H^n(\pi, n; \pi)$ which acts as the identity endomorphism of π .

For $n > 2$, $\eta_0: \pi_n(X) \rightarrow \pi_{n+1}(X)$ is a homomorphism with values in ${}_2(\pi_{n+1}(X))$ and therefore a commutative self-pairing of $\pi_n(X)$ to $\pi_{n+1}(X)$ can be found such that $\alpha \cdot \alpha = \eta_0(\alpha)$. It then follows that $\alpha \beta$ always has order two and therefore $Sq_{n-2}: H^n(\pi_n(X), n; \pi_n(X)) \rightarrow H^{n+2}(\pi_n(X), n; \pi_{n+1}(X))$ is defined and is independent of the particular choice of the self-pairing. Using this pairing, we find

THEOREM 6. If $n > 2$, $Sq_{n-2}(d^n) = k^{n+2}$.

¹ Freudenthal, H., *Comp. Math.*, **5**, 299-314 (1937).

² Fox, R. H., *Bull. Am. Math. Soc.*, **49**, abstract 172 (1943).

³ Hurewicz, W., *Proc. Akad. Amsterdam*, **39**, 117-126 (1936).

⁴ Hopf, H., *Comm. Math. Helv.*, **17**, 307-326 (1945).

⁵ These results depend on Pontrjagin's result that $\pi_{n+2}(S^n) = 0$ for $n \geq 3$. A proof was outlined in *C. R. Acad. Sci. U.R.S.S.*, **19**, 361-363 (1938), but a complete proof has not yet appeared.

⁶ Eilenberg, S., and MacLane, S., *Ann. Math.*, **46**, 489-509 (1945).

⁷ Cf. Eilenberg, S., and MacLane, S., *Proc. Nat. Acad. Sci.*, **32**, 277-280 (1946) for the case $n = 1$.

⁸ Eilenberg, S., and MacLane, S., *Ann. Math.*, **43**, 757-831 (1942).

⁹ Steenrod, N. E., *Ibid.*, **48**, 290-320 (1947).

GROUP THEORETICAL DISCUSSION OF RELATIVISTIC WAVE EQUATIONS

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*Introduction.*¹—The wave functions, ψ , describing the possible states of a quantum mechanical system form a linear vector space V which, in general, is infinite dimensional and on which a positive definite inner product (ϕ, ψ) is defined for any two wave functions ϕ and ψ (i.e., they form a Hilbert space). The inner product usually involves an integration over the whole configuration or momentum space and, for particles of higher spin, a summation over the spin indices.

If the wave functions in question refer to a free particle and satisfy relativistic wave equations, there exists a correspondence between the wave functions describing the same state in different Lorentz frames.

The transformations considered here form the group of all *inhomogeneous* Lorentz transformations (including translations of the origin in space and time). Let $\psi_{l'}$ and ψ_l be the wave functions of the same state in two Lorentz frames l' and l , respectively. Then $\psi_{l'} = U(L)\psi_l$, where $U(L)$ is a linear unitary operator which depends on the Lorentz transformation L leading from l to l' . By a proper normalization, U is determined by L up to a factor ± 1 . (For all details the reader is referred to the paper of reference 2, hereafter quoted as (L).) Moreover, the operators U form a single- or double-valued representation of the inhomogeneous Lorentz group, i.e., for a succession of two Lorentz transformations L_1, L_2 , we have

$$U(L_2 L_1) = \pm U(L_2) U(L_1). \quad (1)$$

Since all Lorentz frames are equivalent for the description of our system, it follows that, together with ψ , $U(L)\psi$ is also a possible state viewed from the original Lorentz frame l . Thus, the vector space V contains, with every ψ , all transforms $U(L)\psi$, where L is any Lorentz transformation.

The operators U may also replace the wave equation of the system. In our discussion, we use the wave functions in the "Heisenberg" representation, so that a given ψ represents the system for all times, and may be chosen as the "Schrödinger" wave function at time 0 in a given Lorentz frame l . To find ψ_{l_0} , the Schrödinger function at time t_0 , one must therefore transform to a frame l' for which $t' = t - t_0$, while all other coordinates remain unchanged. Then $\psi_{l_0} = U(L)\psi$, where L is the transformation leading from l to l' .

A classification of all unitary representations of the Lorentz group, i.e., of all solutions of (1), amounts, therefore, to a classification of all possible relativistic wave equations. Such a classification has been carried out in (L). Two representations $U(L)$ and $\tilde{U}(L) = VU(L)V^{-1}$, where V is a fixed unitary operator, are equivalent. If the system is described by wave functions ψ , the description by

$$\tilde{\psi}_l = V\psi_l \quad (2)$$

is isomorphic with respect to linear superposition, to forming the inner product of two wave functions, and also to the transition from one Lorentz frame to another. In fact, if $\psi_{l'} = U(L)\psi_l$, then $\tilde{\psi}_{l'} = V\psi_{l'} = \tilde{U}(L)\tilde{\psi}_l$. Thus, one obtains classes of equivalent wave equations. Finally, it is sufficient to determine the irreducible representations since any other may be built up from them.

Two descriptions which are equivalent according to (2) may be quite different in appearance. The best known example is the description of the electromagnetic field by the field strength and the four vector potential, respectively. It cannot be claimed either that equivalence in the sense of (2) implies equivalence in every physical aspect. Thus, two equivalent

descriptions may lead to quite different expressions for the charge density or the energy density in configuration space (cf. Fierz,³) because (2) only implies global, but not local, equivalence of the wave functions. It should be emphasized, however, that any selection of one among the equivalent systems or the superposition of non-equivalent systems in any particular way involves an explicit or implicit assumption as to possible interactions, the positive character of densities, etc. Our analysis is necessarily restricted to free particles and does not lead to any assertions about possible interactions.

The present discussion is not based on any hypothesis about the structure of the wave equations provided that they be Lorentz invariant. In particular, it is not necessary to assume differential equations in configuration space. But it is a result of the analysis in (L) that every irreducible wave equation is equivalent (in the sense of (2)) to a system of differential equations. For the relation of the present point of view to other treatments of the subject see reference 11.

In the present note, we shall give, for every representation of (L), a differential equation the solutions of which transform according to that representation. We also will discuss in some detail the infinitesimal operators which generate the irreducible representations determined in (L), and we shall characterize these representations, and hence the covariant differential equations, by certain invariants constructed from the infinitesimal operators. This is of some interest, because the infinitesimal operators are closely related to dynamical variables of the system. L. Gårding⁴ has recently shown that even in the infinite dimensional case one can rather freely operate with infinitesimal transformations. In particular, it immediately follows from his discussion (although it is not explicitly stated in his note) that the familiar commutation rules remain valid.

1. *The Infinitesimal Operators of the Lorentz Group.*—The metric tensor is assumed in the form $g_{44} = 1$, $g_{11} = g_{22} = g_{33} = -1$, $g_{kl} = 0$ ($k \neq l$) and $g^{kl} = g_{kl}$. The scalar product of two four vectors a, b will be denoted by $\{a, b\} = a^k b_k$. Both c , the velocity of light, and \hbar , Planck's constant divided by 2π , are set equal to 1.

The Infinitesimal Operators p_k and M_{kl} . A translation in the x^k -direction is generated by p_k , a rotation in the $(x^k - x^l)$ plane by $M_{kl} = -M_{lk}$ ($k, l = 1, \dots, 4$). These operators are Hermitian, and the unitary operators U which represent the finite Lorentz transformation are obtained by exponentiation; thus $U = \exp(-i\alpha p_k)$ corresponds to a translation by the amount α in the direction x_k . Clearly, p_k are the four momenta of the system, and M_{23}, M_{31}, M_{12} the three components of the total angular momentum. The following commutation rules hold (where $[A, B] = AB - BA$)

$$[M_{kl}, M_{mn}] = i(g_{lm}M_{kn} - g_{km}M_{ln} + g_{kn}M_{lm} - g_{ln}M_{km}), \quad (3a)$$

$$[p_k, p_l] = 0 \quad [M_{kl}, p_m] = i(g_{lm}p_k - g_{km}p_l). \quad (3b)$$

We now define four operators w_k by

$$(w^1, w^2, w^3, w^4) = (v_{234}, v_{314}, v_{124}, v_{321}), \quad (4a)$$

$$v_{k1m} = p_k M_{1m} + p_l M_{mk} + p_m M_{kl} = M_{1m} p_k + M_{mk} p_l + M_{kl} p_m. \quad (4b)$$

Note that w_k is a "pseudo-vector," i.e., it is a vector only with respect to Lorentz transformations of determinant 1. By (3),

$$[M_{kl}, w_m] = i(g_{lm}w_k - g_{km}w_l) \quad [p_k, w_l] = 0. \quad (5)$$

It follows from (3) and (5) that the two operators,

$$P = p^k p_k; \quad W = (1/6)v^{klm}v_{klm} = -w^k w_k, \quad (6)$$

commute with all the infinitesimal operators M_{kl} and p_k . Therefore, they have constant values (i.e., they are multiples of the unit operator) for every irreducible representation of the Lorentz group. (The familiar arguments which establish this for finite dimensional representations can be carried over to the infinite dimensional case. (Cf. V. Bargmann, reference 5, p. 602.))

W may also be written in the form

$$W = 1/2 M_{kl} M^{kl} p_m p^m - M_{km} M^{lm} p^k p_l. \quad (7)$$

(This quantity was first introduced by W. Pauli, cf. Lubanski.⁶) The scalar product $w^k p_k$ vanishes.

2. *Summary of the Results of (L).*—(a) For every irreducible representation the states ψ may be expressed as functions $\psi(p, \xi)$ of the momentum vector p and an auxiliary variable ξ which may assume a finite or an infinite number of values. The momenta p are either all zero, or they vary over the manifold $p^k p_k = P$, with a constant value P . We confine ourselves to the cases in which $p \neq 0$, and either $P > 0$ or $P = 0$, because the remaining cases are unlikely to have direct physical significance.⁵

(b) To every inhomogeneous Lorentz transformation $y^k = \lambda^k_{\alpha} x^{\alpha} + a^k$ (in vector form: $y = \Lambda x + a$) corresponds a unitary operator $U(L)$ defined by

$$U(L)\psi(p, \xi) = e^{-i(a, p)} Q(p, \Lambda) \psi(\Lambda^{-1}p, \xi), \quad (8)$$

where $Q(p, \Lambda)$ is a unitary operator which may depend on p but affects only the variable ξ . The inner product (ϕ, ψ) is obtained by an integration over the manifold $p^k p_k = P$ and by a summation or integration over the variable ξ .

(c) The subgroup of the homogeneous Lorentz transformations which keep a fixed momentum vector p_0 unchanged is called "little group."

(The little groups defined by different vectors p_0 are isomorphic.) The unitary operators $Q(p_0, \Lambda)$ (where $\Lambda p_0 = p_0$) form an irreducible representation of the little group and determine the irreducible representation $U(L)$ of the inhomogeneous Lorentz group.

In all cases the operators M_{kl} have the form $M_{kl} + S_{kl}$, where the

$$M_{kl} = i \left(p_k \frac{\partial}{\partial p^l} - p_l \frac{\partial}{\partial p^k} \right) = i (p_k g_{lj} - p_l g_{kj}) \frac{\partial}{\partial p_j} \quad (9)$$

act on the variables p and correspond to the orbital angular momenta, while the S_{kl} act on the variables ξ and correspond to the spin angular momenta. Both M_{kl} and S_{kl} satisfy the commutation rules (3a). Since the M_{kl} do not contribute to v_{klm} (cf. (4b)), we have

$$v_{klm} = p_k S_{lm} + p_l S_{mk} + p_m S_{kl}; \quad [S_{kl}, p_m] = 0, \quad (10)$$

or, introducing the three-dimensional vector operators,

$$\begin{aligned} \vec{S} &= (S_{23}, S_{31}, S_{12}); & \vec{S}' &= (S_{14}, S_{24}, S_{34}); & \vec{p} &= (p^1, p^2, p^3); \\ \vec{w} &= (w^1, w^2, w^3); & w^4 &= \vec{p} \cdot \vec{S}; & \vec{w} &= \vec{p} \times \vec{S}' - (\vec{p} \times \vec{S}'). \end{aligned} \quad (10a)$$

Clearly, M_{kl} may also be replaced by S_{kl} in the expression (7) for W .

For a fixed momentum vector p_0 the operators w_k are the infinitesimal generators of the little group. Since $w^4 p_k = 0$, only three of them are linearly independent.

3. *Classification of the Irreducible Representations.*—We now turn to a brief summary of the main results, including the characterization of the representations in terms of the operators \vec{p} and \vec{w} . A more detailed discussion will follow in the succeeding sections.

The classes found in (L) (§§ 7, 8) are these:

I. P_s . *Particles of finite mass and spin s .*—Here $P = m^2 > 0$. In the rest system of the particle, the momentum vector has only the one non-vanishing component $p^4 = \pm m$, hence, by (10a), $W = m^2 S^2$. The operator $P^{-1}W$ represents the square of the spin angular momentum, and has the value $s(s+1)$ ($s = 0, 1/2, 1, \dots$) for an irreducible representation. For a given momentum vector there are $2s+1$ independent states. The representation $U(L)$ is single or double valued according to whether s is integral or half integral. The lowest cases ($s = 0, 1/2, 1$) correspond to the Klein-Gordon, Dirac and Proca equations, respectively.

II. Q_s . *Particles of zero rest mass and discrete spin.*—These representations may be considered limiting cases of the representations P_s for $m \rightarrow 0$. Then both P and W are equal to zero, and do not suffice to characterize these representations. For a given momentum vector, there exist 2 independent states if $s \neq 0$ (corresponding to two different states of polariza-

tion), and there is only one state if $s = 0$. Right and left circularly polarized states are described by the operator equations $w_k = sp_k$, and $w_k = -sp_k$, respectively, so that the representation 0_s is characterized by $P = 0$, $w_k w_l = s^2 p_k p_l$. The lowest cases ($s = 0, 1/2, 1$) correspond to the scalar wave equation, the neutrino equation, and Maxwell's equations, respectively.

III. $0(\Xi)$ and $0'(\Xi)$. *Particles of zero rest mass and continuous spin.*—Here, $P = 0$, $W = \Xi^2$, where Ξ is a real positive number. For a given momentum vector there exist infinitely many different states of polarization, which may be described by a continuous variable. The representation $0(\Xi)$ is single valued, while $0'(\Xi)$ is double valued.

To construct these representations explicitly, we shall select, in each case, one among the equivalent sets of wave equations, define a Lorentz invariant inner product (ϕ, ψ) , and prove the operator relations stated above. We shall operate in momentum space; this is particularly simple, because the momenta (but not the coordinates) are defined by the Lorentz group, as infinitesimal translations.

4. *The Class P_s .*—(a) $s = 0$. Here, the variable ξ assumes only one value and may therefore be omitted. Consequently, $Q(p, \Lambda) = 1$ (cf. reference 8), and for the little group the trivial one-dimensional representation is obtained. Hence, $S_{kl} = 0$, and $w_k = 0$. The wave equation reduces to $p^k p_k = m^2$; the inner product (ϕ, ψ) is determined by the norm (ψ, ψ) of a wave function,

$$(\psi, \psi) = \int |\psi(p)|^2 d\Omega, \text{ where } d\Omega = |p^4|^{-1} dp^1 dp^2 dp^3, \quad (11)$$

the integral being extended over both sheets of the hyperboloid $p^k p_k = P = m^2$. The expression (11) is Lorentz invariant, because $d\Omega$ is an invariant volume element in momentum space. For the wave function in configuration space, one finds

$$\psi(x) = (2\pi)^{-1/2} \int e^{-i(p, x)} \psi(p) d\Omega, \quad (12)$$

where x stands for x^1, x^2, x^3, x^4 . It is well known that (ψ, ψ) cannot be simply expressed in configuration space, because for the Klein-Gordon equation the density is indefinite, and the integral over the density in configuration space coincides with (11) only if $\psi(p) = 0$ whenever $p^4 < 0$.

(b) $s = 1/2 N$ with $N = 1, 2, 3, \dots$. For particles of higher spin we use the equations first derived by Dirac⁷ in the form essentially given in reference 8. We use for ξ the N four-valued variables ξ_1, \dots, ξ_N in which the wave function $\psi(p; \xi_1, \dots, \xi_N)$ is symmetric. We define for every ξ , four-dimensional matrices γ_r^k of the same nature as are used in Dirac's electron theory:

$$\gamma_r^k \gamma_r^l + \gamma_r^l \gamma_r^k = 2g^{kl} \quad (k, l = 1, 2, 3, 4). \quad (13)$$

The γ with different lower indices ν commute. The $\gamma^1, \gamma^2, \gamma^3$ are skew Hermitian, γ^4 is Hermitian. The wave equations then are

$$\gamma_\nu^k p_k \psi = m\psi \quad (\nu = 1, 2, \dots, N). \quad (14)$$

It follows from any of these equations in well known fashion that

$$g^{kl} p_k p_l \psi = p^k p_k \psi = m^2 \psi. \quad (14a)$$

The infinitesimal operators of displacement are the p , those of four-dimensional rotation the $M_{kl} = M_{kl} + S_{kl}$ with M_{kl} of (9) and

$$S_{kl} = \frac{1}{2i} \sum_\nu \gamma_{\nu k} \gamma_{\nu l} \quad (k \neq l), \quad (15)$$

where the

$$\gamma_{\nu k} = g_{kl} \gamma_\nu^l \quad (15a)$$

satisfy the same relations (13) as do the γ_ν^k .

The invariant scalar product is

$$(\psi, \psi) = \int \left| \sum_\xi \psi^* \gamma_1^4 \gamma_2^4 \dots \gamma_N^4 \psi \right| d\Omega. \quad (16)$$

In fact, (16) is invariant both with respect to the operators M_{kl} and also with respect to the S_{kl} . The latter condition means that

$$((1 + i \epsilon S_{kl})\psi, (1 + i \epsilon S_{kl})\psi) = (\psi, \psi),$$

up to terms with ϵ^2 . This formula can be verified by observing that, if both k and l are space like S_{kl} is a Hermitian matrix and commutes with the product of the γ^4 . If either k or l is 4, S_{kl} is skew Hermitian, but anti-commutes with the product of the γ^4 . It follows that (16) is invariant with respect to the proper Lorentz transformations. Its invariance with respect to reflections, etc., can also be shown.

The absolute sign in (16) is necessary to make it positive definite. We now shall give (16) a new form which is based on the set of identities

$$(p_4)^\nu \gamma_\nu^4 \dots \gamma_2^4 \gamma_1^4 \psi = m^\nu \psi + A_\nu \psi, \quad (17)$$

where A_ν is a skew Hermitian matrix involving only the first ν of the γ^k (and the p). We can prove (17) best by induction: applying $p_4 \gamma_{\nu+1}^4$ to (17) gives, by means of (14),

$$\begin{aligned} (p_4)^{\nu+1} \gamma_{\nu+1}^4 \gamma_\nu^4 \dots \gamma_2^4 \gamma_1^4 \psi &= m^\nu p_4 \gamma_{\nu+1}^4 \psi + p_4 \gamma_{\nu+1}^4 A_\nu \psi \\ &= m^{\nu+1} \psi + (-m^\nu p_k \gamma_{\nu+1}^k + p_4 \gamma_{\nu+1}^4 A_\nu) \psi \quad (k = 1, 2, 3). \end{aligned} \quad (17a)$$

The last bracket is $A_{\nu+1}$: it is skew Hermitian and involves only the first $\nu + 1$ of the γ so that (17) is established by induction. Setting $\nu = N$ in (17), multiplying with ψ and summing over the ξ yields

$$p_4^N \sum_{\zeta} \psi^* \gamma_1^4 \gamma_2^4 \dots \gamma_N^4 \psi = m^N \sum_{\zeta} |\psi|^2 + \sum_{\zeta} \psi^* A_N \psi. \quad (17b)$$

Because of the skew Hermitian nature of A_N , the last term is imaginary. Since the two other terms of (17b) are real, they must be equal. As a result, we can write for (16) also

$$(\psi, \psi) = \int |m/p_4|^N \sum_{\zeta} |\psi|^2 d\Omega. \quad (18)$$

At the same time, (18) permits us to give another form to the scalar product,

$$(\psi, \psi) = \int |p_4|^{-N-1} \sum_{\zeta} |\psi|^2 dp_1 dp_2 dp_3, \quad (18a)$$

which differs from (18) or (16) by the positive constant m^{-N} . It may be worth noting here that the absolute signs in (16), and in the definition (11) of $d\Omega$ (or in (18a)), can be omitted in case of an odd N . This makes it possible to define a simple positive definite scalar product in coördinate space by means of (12). In particular, for $N = 1$, (16) (or (18a)) equals the integral of $|\psi|^2$ over ordinary space. In case of even N (integer spin s) no simple positive definite scalar product can be defined in coördinate space.

It is now established that the solutions of (14) form a Lorentz invariant set in which a positive definite scalar product (16) or (18a) can be defined. We shall now determine the representation of §2 to which the solutions belong and will also calculate the invariants P and W .

In order to define a little group, we choose as momentum p_0 with the components $0, 0, 0, m$. The little group then becomes the group of rotations in ordinary space. If we assume that the γ^4 are diagonal, with diagonal elements $1, 1, -1, -1$, equation (14) shows that only those components of ψ can be different from zero which correspond to the first two rows of γ_ν . There are 2^N such components, the rest of the 4^N components of ψ must vanish. Even these components will not be independent: as a result of the symmetry of the ψ in the ζ , all components of ψ will be equal in which the same number κ of the N indices ζ correspond to the first row of the γ , the $N - \kappa$ other indices to the second row. Since κ can assume any of the values between 0 and N , there are $N + 1$ such components. If $p_4 = -m$, the same considerations will hold, except that the last two rows of γ will play the rôle which the first two rows play in case of $p_4 = m$.

In order to determine the transformation properties of these $N + 1 = 2s + 1$ independent components under the elements of the little group, we note that the space like M give zero if applied to ψ with a purely time like $p = p_0$. We need only to calculate, therefore, the effect of the S_{kt} .

on ψ . Since, in particular, $\frac{1}{2}i\gamma_1\gamma_2$ commutes with γ^4 , but is not identical with it, we can assume that it is diagonal and has the diagonal elements $\frac{1}{2}, -\frac{1}{2}, \frac{1}{2}, -\frac{1}{2}$. If the sum of such $\frac{1}{2}i\gamma_1\gamma_2$ is applied to the component of ψ in which κ of the ξ_ν correspond to the first row, $N - \kappa$ to the second row, this component will be multiplied by $\frac{1}{2}\kappa - \frac{1}{2}(N - \kappa) = \kappa - s$. Since κ runs from 0 to $N = 2s$, the $M_{12}\psi = S_{12}\psi$ will run from $-s\psi$ to $s\psi$. Hence the representation of the little group in question is $D^{(s)}$, as was postulated.

Because of (10a), W becomes $m^2(S_{23}^2 + S_{31}^2 + S_{12}^2)$ or, since the S_{23} , S_{31} , S_{12} are the infinitesimal operators of $D^{(s)}$, we have $W = m^2s(s + 1)$ as given⁹ in §3. The value of P is m^2 because of (14a).

5. *The Class O_s .*—(a) $s = 0$. The corresponding discussion in the preceding section may be literally applied to this case, with the exception that $m = 0$ and that the integral (11) is to be extended over the light cone.

(b) The wave equations can be obtained by setting $m = 0$ in (14). The infinitesimal operators continue to be given by (9) and (15). The scalar product must be defined by (18a) because (16) vanishes for all ψ . The invariance of this scalar product follows from the invariance of (18a) for finite mass because, except at $p_1 = p_2 = p_3 = 0$, the wave function is continuous in m .

The essential difference between finite and zero mass is that, in the latter case, not only the infinitesimal operators but also the wave equation are invariant under any one of the operators $\Gamma_\nu = i\gamma_\nu^1\gamma_\nu^2\gamma_\nu^3\gamma_\nu^4$. As a result, for $m = 0$, the linear manifold defined by (14) can be decomposed into invariant manifolds by giving definite values to the Γ_ν . In particular, we shall be concerned henceforth with the manifold defined by (14) and

$$\Gamma_\nu\psi = \psi \quad (\nu = 1, 2, \dots, N), \quad (19a)$$

and with the other one for which

$$\Gamma_\nu\psi = -\psi \quad (\nu = 1, 2, \dots, N) \quad (19b)$$

holds. Both manifolds are invariant under proper Lorentz transformations but go over into each other by reflections: they correspond physically to right and left circular polarization.¹⁰

Let us now again choose a particular momentum vector p_0 in order to define the little group. The covariant components of p_0 shall be 0, 0, 1, 1. The wave equations (14) then can be written, after multiplication with γ_ν^4 , in the form

$$\gamma_\nu^3\gamma_\nu^4\psi = \psi \quad (\nu = 1, 2, \dots, N). \quad (20)$$

It is now advantageous to assume that the $\gamma^3\gamma^4$ are diagonal, their diagonal elements being 1, 1, -1, -1. Equation (20) then expresses the fact that ψ for the p_0 in question is different from zero only if all ξ have values corre-

sponding to the first two rows of the γ . Since the Γ commute with the $\gamma^4\gamma^3$ but are not identical with them, they may be also assumed to be diagonal, with diagonal elements 1, -1, 1, -1. Hence, in the manifold defined by (20) and (19a) all components of ψ vanish (for $p = p_0$) unless all ξ have values corresponding to the first rows of the γ : the manifold (20), (19a) is one dimensional for given p . The same holds for the manifold defined by (20), (19b) except that in this case $\psi(p_0; \xi_1, \dots, \xi_N)$ differs from zero only if all ξ have values corresponding to the second row of the γ . For given momentum, ψ has only two independent components.

The infinitesimal operators of the little group are $M_{12}, M_{13} - M_{14}, M_{23} - M_{24}$ which leave p_0 invariant. The corresponding M give again zero if applied to ψ at $p = p_0$. The S corresponding to the second of the above operators (cf. (15), (15a)) is a sum of matrices $1/2i(\gamma_\nu^1\gamma_\nu^3 + \gamma_\nu^1\gamma_\nu^4)$. It vanishes if applied to our ψ as can be seen by applying $\gamma_\nu^1\gamma_\nu^3$ to (20). The same holds for $M_{23} - M_{24}$. On the other hand, $1/2i\gamma_\nu^1\gamma_\nu^2$ gives $1/2\psi$ if applied to the ψ of (20), (19a), and gives $-1/2\psi$ if applied to the ψ of (20), (19b). One sees this most easily by applying $1/2i\gamma_\nu^1\gamma_\nu^2$ to (20) and making use of (19). As a result, $M_{12}\psi = \pm 1/2N\psi = \pm s\psi$ for the two manifolds in question: these indeed belong to the representation 0_s of the inhomogeneous Lorentz group.

The value of the invariant P is zero. The above also involves a calculation of the w for the ψ at $p = p_0$: we have $w^3\psi = M_{12}\psi = \pm s\psi$, $w^1\psi = (M_{12} + M_{23})\psi = 0$, $w^2\psi = (M_{31} + M_{14})\psi = 0$, $-w^4\psi = M_{12}\psi = \pm s\psi$. It follows that the value of the second invariant $W = -(w^4)^2 + (w^1)^2 + (w^2)^2 + (w^3)^2$ is also zero for all the manifolds 0_s ; these cannot be characterized by P and W . However, these manifolds can be characterized by the equation $P = 0$ with the additional set

$$w_k = sp_k \text{ and } w_k = -sp_k, \quad (21)$$

the $+$ applying to (19a), the $-$ to (19b). Both these equations are invariant with respect to proper Lorentz transformations. If reflections are to be included, one can combine them into $w_k w_l = s^2 p_k p_l$.

6. *The Class $0(\Xi)$.*—Here, the auxiliary variable is a space like four vector ξ of length l , orthogonal to p . The scalar function $\psi(p, \xi)$ is determined by the equations¹¹

$$g^{kl}p_k p_l \psi = 0; g^{kl}p_k \xi_l \psi = 0; g^{kl}\xi_k \xi_l \psi = -\psi, \quad (22)$$

$$p_k \partial \psi / \partial \xi_k = -i\Xi \psi, \quad (22a)$$

with a real positive constant Ξ . By (22a), for every real number ρ ,

$$\psi(p, \xi + \rho p) = e^{-i\rho\Xi} \psi(p, \xi). \quad (23)$$

The infinitesimal operators of displacement are the p_k , those for rotations are the M of (9) plus the

$$S_{kl} = i \left(\xi_k \frac{\partial}{\partial \xi^l} - \xi_l \frac{\partial}{\partial \xi^k} \right) = i (\xi_k g_{lj} - \xi_l g_{kj}) \frac{\partial}{\partial \xi_j}. \quad (24)$$

In order to find the invariant scalar product, we introduce, for every vector p on the light cone, two real space like vectors $u^{(1)}(p)$ and $u^{(2)}(p)$ of length one, orthogonal to p and to each other, so that

$$\{u^{(r)}(p), p\} = 0, \quad \{u^{(r)}(p), u^{(s)}(p)\} = -\delta_{rs} \quad (r, s = 1, 2). \quad (25)$$

Then ξ is a linear combination of p , $u^{(1)}(p)$, $u^{(2)}(p)$,

$$\xi = \alpha p + \beta_1 u^{(1)}(p) + \beta_2 u^{(2)}(p), \quad (26)$$

where α and the β are real. $\{\xi, \xi\} = -1$ implies $\beta_1^2 + \beta_2^2 = 1$, hence $\beta_1 + i\beta_2 = e^{i\tau}$ with a suitable real angle τ . $\psi(p, \xi)$ is therefore a function of p , α , τ ,

$$\psi(p, \xi) = \phi(p, \alpha, \tau). \quad (27)$$

The choice of the $u^{(r)}(p)$ is, of course, not unique. Let $v^{(r)}(p)$ be another system of vectors which satisfy (25). They may be expressed in the form (26), i.e.,

$$v^{(r)}(p) = \kappa_r p + \sum_s \lambda_{sr} u^{(s)}(p) \quad (r, s = 1, 2).$$

By (25), the matrix λ_{sr} is orthogonal. In terms of the $v^{(r)}$, $\xi = \alpha' p + \sum_r \beta'_r v^{(r)}(p)$, where $\beta'_r = \sum_s \lambda_{sr} \beta_s$. In particular

$$\beta'_1 + i\beta'_2 = e^{i\tau'}; \quad \tau' = \tau + \lambda \quad (28)$$

λ depending on the λ_{sr} . By (23), $|\phi(p, \alpha, \tau)| = |\phi(p, 0, \tau)|$, and we define the norm of ψ by

$$(\psi, \psi) = \int |\phi(p, 0, \tau)|^2 d\Omega d\tau. \quad (29)$$

This expression is independent of the choice of the $u^{(r)}$. In fact, let $\phi(p, \alpha, \tau) = \phi'(p, \alpha', \tau')$ where the primed variables refer to another set $v^{(r)}$. Then $|\phi(p, 0, \tau)| = |\phi'(p, \alpha', \tau')| = |\phi(p, 0, \tau')|$, and $|d\tau'/d\tau| = 1$. To prove the Lorentz invariance of (29) we proceed as follows: If a homogeneous Lorentz transformation maps p on $\Lambda^{-1}p$, and ξ on $\Lambda^{-1}\xi$, we may, in particular, choose the $u^{(r)}(p)$ in the new system to be the transforms of the original ones; then the coefficients α , β_1 , β_2 in (26), and hence τ remain unchanged, and the integral (29) is invariant.

If we choose as the basic vector again p_0 with the components 0, 0, 1, 1 the infinitesimal operators of the little group are again M_{12} , M_{13} and M_{14} .

$M_{23} - M_{24}$. The M parts of these give zero for $p = p_0$, the S parts of the latter two are

$$S_{13} - S_{14} = -i\xi_1 \left(\frac{\partial}{\partial \xi_3} + \frac{\partial}{\partial \xi_4} \right) + i(\xi_3 - \xi_4) \frac{\partial}{\partial \xi_1}, \quad (30a)$$

$$S_{23} - S_{24} = -i\xi_2 \left(\frac{\partial}{\partial \xi_3} + \frac{\partial}{\partial \xi_4} \right) + i(\xi_3 - \xi_4) \frac{\partial}{\partial \xi_2}. \quad (30b)$$

Because of (22a), the first term gives, if applied to ψ at $p = p_0$ just $\Xi\xi_1\psi$ and $\Xi\xi_2\psi$, respectively. The second terms vanish because of the second equation of (22). Hence ψ is not invariant under the "displacements" $M_{13} - M_{14}$ and $M_{23} - M_{24}$ in ξ space, and the sum of the squares of the "momenta" is $(\xi_1^2 + \xi_2^2) \Xi^2 = \Xi^2$ because of the last equation of (22). This is also the value of W , while $P = 0$.

7. *The class 0' (Ξ).*—Since the discussion of this last case follows the pattern of the preceding section we confine ourselves to stating the main results. We introduce, in addition to the vector ξ , a discrete spin variable ζ which can assume four values. The wave equations become

$$\gamma^k p_k \psi = 0; \quad g^{kl} p_k \xi_l \psi = 0; \quad g^{kl} \xi_k \xi_l \psi = -\psi. \quad (31)$$

$$p_k \partial \psi / \partial \xi_k = -i\Xi \psi \quad (31a)$$

The parameters α and τ are introduced as before. The norm is given by

$$(\psi, \psi) = \int p_4^{-2} \sum |\phi(p, 0, \tau)|^2 dp_1 dp_2 dp_3 d\tau. \quad (32)$$

(Cf. (18a) and (29).) Again $W\psi = \Xi^2\psi$, $P\psi = 0$.

It may be remarked that the scalar product has a simple positive definite form in coordinate space for these equations.¹¹

¹ All the essential results of the present paper were obtained by the two authors independently, but they decided to publish them jointly.

² Wigner, E. P., *Ann. Math.*, **40**, 149-204 (1939).

³ Fierz, M., *Helv. Phys. Acta*, **XII**, 3-37 (1939).

⁴ Gårding, L., *Proc. Nat. Acad. Sci.*, **33**, 331-332 (1947).

⁵ Gelfand, L., and Neumark, M., *J. Phys. (USSR)*, **X**, 93-94 (1946); Harish-Chandra, *Proc. Roy. Soc. (London)*, **A**, **189**, 372-401 (1947); and Bargmann, V., *Ann. Math.*, **48**, 568-640 (1947), have determined the representations of the homogeneous Lorentz group. These are representations also of the inhomogeneous Lorentz group. In the quantum mechanical interpretation, however, all the states of the corresponding particles are invariant under translations and, in particular, independent of time. It is very unlikely that these representations have immediate physical significance. In addition, the third paper contains a determination of those representations for which the momentum vectors are space like. These are not considered in the present article as they also are unlikely to have a simple physical interpretation.

⁶ Lubanski, J. K., *Physica*, **IX**, 310-324 (1942).

⁷ Dirac, P. A. M., *Proc. Roy. Soc., A*, **155**, 447-459 (1936).

⁸ The literature on relativistic wave equations is very extensive. Besides the papers quoted in reference 11, we only mention the book by de Broglie, L., *Théorie générale des particules à spin* (Paris, 1943), and the following articles which give a systematic account of the subject: Pauli, W., *Rev. Mod. Phys.*, **13**, 203-232 (1941); Bhabha, H. J., *Rev. Mod. Phys.*, **17**, 203-209 (1945); Kramers, H. A., Belinfante, F. J., and Lubánski, J. K., *Physica*, **VIII**, 597-627 (1941). In this paper, the sum of (14) over all ν was postulated; (14a) then has to be added as an independent equation (except for $N = 1$). Reference 11 uses these equations in the form given by Kramers, Belinfante and Lubánski.

⁹ One may derive this result in a more elegant way, without specializing the coördinate system. For the sake of brevity, we omit this derivation.

¹⁰ de Wet, J. S., *Phys. Rev.*, **58**, 236-242 (1940), in particular, p. 242.

¹¹ Wigner, E. P., *Z. Physik*, (1947).

STEREOSCOPIC ACUITY FOR VARIOUS LEVELS OF ILLUMINATION*

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Several experiments have demonstrated that the threshold for stereoscopic vision is influenced by certain important variables (see the review by Graham¹), but little attention has been paid to the systematic exploration of parameters (e.g., intensity and wave-length) which are known to be important for other visual functions.² The present report gives data on one of those variables, intensity of "white" light, as it influences the threshold for stereoscopic vision.

Apparatus.—Two 300-watt Mazda bulbs are used as light sources, one for each eye. The light sources are fastened to a movable wooden stand which may be placed in either of two positions, thus allowing for a small range of intensity variation. Additional adjustment of intensity may be achieved by inserting filters of various densities in a holder adjacent to the light source for each eye.

The two filter holders are attached to a pair of metal funnels, 4 inches in diameter and $3\frac{3}{4}$ inches in length; the funnels in turn are fastened to the outer wall of the dark room in which the subject sits.

A piece of opal glass, fastened to the inner wall of the dark room and in front of the funnels, diffuses the light from the two bulbs. A piece of masonite, containing two holes of $3\frac{1}{2}$ inch diameter, is mounted in front of the opal glass. These holes expose two photographic plates which are fitted into slots in the masonite and on which the reticles of the two visual fields are photographed. Both test fields contain three vertical reticle marks, each with a width of 20 minutes and a height of two degrees of visual angle. The reticle marks are equidistant from one another at a

separation of 4.08 degrees. The central mark is centered in the visual field.

On the front side of the piece of masonite, a vertical target (long vertical line) is permanently fastened over the left hold and to the left of the central reticle mark. The vertical target on the right side, on the contrary, is movable and may be adjusted to the left or the right of its central position by means of a micrometer. Both of the targets have a width of 20 minutes and a length of 19 degrees of visual angle. The micrometer, which the subject uses for adjusting the position of the target, is connected with a metal pointer through the side wall of the dark room; the rotation of the pointer may be read on a degree scale by the experimenter. Each degree of rotation moves the right target through 0.95 second of arc.

A front-silvered mirror is placed five inches in front of each reticle plate to form an angle of 45 degrees with the optical path. A second front-silvered mirror, in both the left and right eye systems, is placed on a movable platform four inches from the first mirror. Each of the latter mirrors also forms an angle of 45 degrees with its optical paths. The two platforms are attached to two eyepieces whose separation may be adjusted to the interpupillary distance of the subject.

All of the above apparatus is enclosed in a large box from which the eyepieces project. Each eyepiece has an aperture of $\frac{3}{8}$ inch diameter and holds a convex lens, the focal length of which (14 inches) equals the distance from the lens to the reticle.

The dark room in which the subject is seated is painted a dull black and is completely light tight.

Procedure.—At the start of each experimental session, the subject was allowed to dark adapt for 25 minutes. During the first 15 minutes of this period the subject wore goggles which transmitted the far red region of the spectrum;³ the last ten minutes were spent in the completely darkened room.

Two subjects were used and each provided three complete sets of data. Both subjects were highly experienced in making stereoscopic settings. Each set of data for each subject consisted of 20 readings taken at each of ten intensity levels ranging from -4.04 log millilamberts to 2.27 log millilamberts. The intensity levels were presented to the subject in order of increasing magnitude and two minutes of light adaptation were given at each level. A rest period, 10 to 20 minutes in length, was given between the fifth and sixth intensity levels in each session.

For each reading the subject started from a randomly selected micrometer position and adjusted the movable target, turning the micrometer until the fused target line appeared in the same plane as the three reticle lines. Both subjects used a "bracketing" procedure in making settings.

The position of the movable target at each adjustment was recorded by the experimenter from the scale on the outside wall of the dark room. Readings at each intensity level were made at the rate of about 4 per minute.

If s_l be the separation between the reference reticle line and target for the left eye and s_r the corresponding separation in the right eye, then the difference, d (i.e., $s_l - s_r$), divided by R , the distance from the lens to the plane of the target and reticles, gives the angular disparity, η , in radians.¹ For threshold,

$$\eta_t = 206,265 \frac{d_t}{R} \quad (1)$$

in seconds of arc, where d_t and R are measured in the same units. In the present experiment, d_t is defined as the average deviation of the "equality" settings.

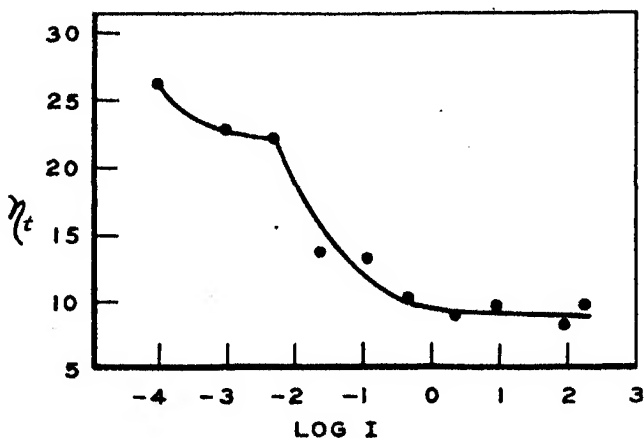


FIGURE 1

The threshold for stereoscopic vision in seconds as a function of field brightness in millilamberts.

Results.—The results⁴ of the experiment are presented in figure 1. The data show that η_t decreases (stereoscopic acuity increases) as light intensity increases, until at high intensities, the curve approaches a final limiting value. The mean of the average deviations (representing 120 observations at each intensity) undergoes a threefold change as intensity is changed by a factor of one million. At low intensities the results indicate the usual discontinuity of rod and cone functions.² The line drawn through the data is a theoretical curve based on an extension of the Hecht and Mintz⁵ formulation of the photochemical basis of visual acuity.

Our data provide some slight indication that the mean setting also varies with the intensity level. The mean settings show considerable variability

but the trend is in the direction of "nearer" mean settings with an increase in intensity.

Discussion.—A theory of stereoscopic vision in terms of its relation to other visual functions is relatively undeveloped.

In experiments on monocular visual acuity for thin lines, Hecht and Mintz⁶ found that their results could be accounted for by assuming that the threshold visual angle subtended by the thin lines is proportional to a threshold brightness difference. Their development involved setting the threshold visual angle, α , proportional to $\Delta I/I$ and substituting α in the theoretical solution for $\Delta I/I$ as a function of intensity. The procedure leads to the expression:

$$\alpha = b \left[1 + \frac{1}{(KI)^{1/4}} \right]^2, \quad (2)$$

where α is the threshold visual angle for resolution of thin lines; b and K are constants; and I is light intensity. (For a different theoretical treatment of other acuity figures see Shlaer⁶ and Shlaer, Smith and Chase.⁷)

A first approximate extension of visual acuity theory to the stereoscopic situation may proceed from the Hecht and Mintz work.⁶ We may assume that threshold differences in brightnesses in some part of the two fields provide one of the bases for the discrimination of differences in depth. Therefore η_t may be considered proportional to $\Delta I/I$ and a solution for η_t may be obtained in a manner similar to that followed by Hecht and Mintz in determining α . Such a treatment obviously leads to a formula of the same form as (2) above. The data and theoretical curve are shown in figure 1.

Data available at the present time do not seem to warrant a more elaborate theoretical discussion, but the extent of agreement between this particular extension of Hecht's theory and the present measurements of stereoscopic acuity suggests the need for more extensive study of parameters of stereoscopic acuity.

Summary.—(1) Measurements of stereoscopic acuity were made at ten levels of intensity ranging from -4.04 to 2.27 log millilamberts. (2) The average deviations of the "equality" settings is taken to be the measure of the minimum resolvable difference angle, η_b , for stereoscopic acuity. η_t is large at low intensities and decreases at high intensities. (3) The typical "rod-cone" discontinuity characteristic of other visual functions is present in the data for stereoscopic acuity. Discrimination of depth seems to be possible at intensities below cone threshold. (4) The results are discussed in terms of theories of brightness discrimination and visual acuity.

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¹ Graham, C. H., "Visual Perception." In *Handbook of Experimental Psychology* (edited by Stevens). In preparation.

² Hecht, S., *Physiol. Rev.*, **17**, 239 (1937).

³ Miles, W. R., *Fed. Proc.*, **2**, 109 (1943).

⁴ The data for the figure are represented by the following pairs of numbers. The first number of each pair is the logarithm of the intensity (in millilamberts); the second the mean of the 6 average deviations (in seconds of arc). 2.27, 9.78; 1.96, 8.34; 0.96, 9.66; 0.36, 9.06; -0.34, 10.43; 0.94, 13.37; -1.64, 13.67; -2.34, 22.17; -3.04, 22.82; -4.04, 26.08.

⁵ Hecht, S., and Mintz, E. U., *J. Gen. Physiol.*, **22**, 593 (1939).

⁶ Shlaer, S., *J. Gen. Physiol.*, **20**, 165 (1937).

⁷ Shlaer, S., Smith, E. L., and Chase, A. M., *J. Gen. Physiol.*, **25**, 553 (1942).

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THE EFFECT OF IRRADIATION ON RECOMBINATION IN *ESCHERICHIA COLI*

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Gates¹ has reported that the growth processes of *Escherichia coli* are less sensitive to ultra-violet light than cell division. When these organisms are exposed to a limited dose of radiation, they form "spaghetti-like" filaments which attain lengths up to 150 microns and diameters up to 3 times that of normal cells. We have examined these filaments both in the living state with the phase microscope and after staining the nuclear bodies by Robinow's procedure.² As the filaments grow it was observed that the nuclear bodies divide regularly and each filament soon contains pairs located 3 to 4 microns apart. After several hours many of the filaments recover from the effect of the ultra-violet treatment and produce normally dividing cells of the organism. In studying the mechanism of this recovery, it was observed that in some filaments a swelling appears near the center and the nuclear bodies migrate to this area and apparently fuse together to form a mass of Feulgen-positive material. The swelling increases in size until it is 6-10 microns in diameter. The nuclear material takes on a granular appearance and a colony of apparently normal organisms will develop at this site on agar plates seeded with irradiated organisms. The appearance of the process is analogous to that observed by Dienes³ with cultures of a *Proteus* species. It seems possible that the organism, unable to divide normally, has resorted to a primitive or rudimentary sexual mechanism. We are aware of the pitfalls inherent in cytological studies of microorganisms, and since our photographs were not convincing we have used a cultural procedure for studying the effect of ultra-violet radiation on sexual activity.

A number of *E. coli* mutants were obtained from Dr. Lederberg.⁴ These strains are unable to synthesize certain necessary amino acids or vitamins

and, therefore, will not grow in a minimal medium. However, when two strains with different deficiencies are grown together one can isolate from the resultant culture individuals which are prototrophs of the parent strain (i.e., organisms which are identical with the parent strain in their nutritional requirements). Other mutations such as virus resistance or inability to ferment lactose can be tested at the same time. These are distributed in the prototrophs isolated from mixed cultures of mutant strains as recombinations are distributed in higher forms. This suggests that *E. coli* has a life cycle like *Neurospora* with only an occasional sexual phase.

Four-hour broth cultures of the mutant strains were exposed to ultra-violet lamp for 30 seconds and inoculated singly and in combinations into nutrient broth along with similar unirradiated controls. After the cultures were incubated for 6-24 hours, they were washed and plated on minimal medium to detect prototrophs. It will be observed from table 1 that when two strains were grown together which were both B⁻M⁻ (biotin and methionine deficient) in no case did one obtain organisms which could grow on the minimal medium. A similar result occurred when two strains of T⁻L⁻B₁⁻ (threonine, leucine and thiamin deficient) organisms were grown together. When two strains with different deficiencies, for example, B⁻M⁻ (which can synthesize threonine, leucine and thiamin) was grown with T⁻L⁻B⁻ (which can synthesize biotin and methionine) organisms were obtained which could synthesize all factors (prototrophs of the parent strain) in numbers of about 1 for each 10⁷ cells plated as Lederberg⁴ has reported. It will be observed from table 1 that the organisms subjected to irradiation produced up to 17 times as many prototrophs as the unirradiated cultures. Yet the irradiation produced no change in the cultures inoculated with single strains or with pairs of strains containing the same deficiencies.

TABLE 1
EFFECT OF IRRADIATION ON RECOMBINATIONS

ORGANISMS*	IRRADIATED INOCULUM			CONTROL INOCULUM		
	TOTAL COUNT	PROTO-TROPHS	RATE/MILLION	TOTAL COUNT	PROTO-TROPHS	RATE/MILLION
58-161 + Y10	50M	130	2.6	280	230	0.8
58-161 + Y53	21	160	7.6	220	100	0.45
58-161 + Y87	60	0	0	270	0	0
Y53 + Y10	80	0	0	190	0	0
Y53 + Y87	70	360	5.1	250	100	0.4
Y87 + Y10	90	330	3.7	220	130	0.6
58-161	70	0	0	150	0	0
Y53	50	0	0	340	0	0
Y10	70	0	0	160	0	0
Y87	110	0	0	120	0	0

* 58-161 = B⁻M⁻; Y10 = T⁻L⁻B₁⁻; Y53 = T⁻L⁻B₁⁻-Lac⁻; Y87 = B⁻M⁻-Lac⁻.

The young inocula of the 4 strains were washed carefully and pipetted both singly and in combinations onto plates of minimal agar in dilutions of 1-10 and 1-1,000,000. The total inoculum in each case was suspended in 1 ml. of saline which left a film of moisture on top of the agar in which the organisms could move freely. Some of the seeded plates were exposed to ultra-violet light for the periods of time indicated in table 2. Then all plates were placed at 37°C. for two hours at which time some filaments were formed in the plates irradiated for the longest period. At that time another layer of minimal agar was poured on the plates of the 1-10 dilution and nutrient agar was poured on the plates containing the 1-1,000,000 dilution. After incubation the latter plates gave the total numbers of organisms involved and the former gave a measure of the number of recombinations which had occurred. It will be observed that the maximum production of prototrophs occurs with about 10 seconds exposure to ultra-violet under the conditions of our experiment but that even two seconds exposure enhanced the rate of occurrence markedly.

TABLE 2
EFFECT OF TIME OF IRRADIATION OF SEEDED PLATES ON RECOMBINATIONS

ORGANISMS	PROTOTROPHS PER MILLION CELLS ON PLATES IRRADIATED FOR (SECONDS):				
	0	2	5	10	30
58-161 + Y53	0.45	2.5	4.4	5.6	3.2
Y53 + Y87	0.8	3.2	4.1	6.8	7.3
58-161 + Y10	0	0	0	0	0
58-161 + Y87	0	0	0	0	0
58-161	0	0	0	0	0

From a number of plates, colonics were isolated and transferred to cosin-methylene blue agar to determine lactose fermentation. Of the 25 organisms tested from the prototrophs obtained from the combination of two lactose negative strains, no lactose positive organisms were found from either the normal or the irradiated inoculum. From the combination of a lactose positive with a lactose negative strain 28% of the prototrophs were lactose positive from both the control and the irradiated inoculum. These data strongly suggest that the small amounts of irradiation stimulate a sexual mechanism in bacteria as measured by the increased rate of recombination. Whether this stimulates a sexual conjugation or the "crossing-over" after such conjugation, or merely the production of a transforming principle is not evident from these experiments.

TABLE 3
EFFECT OF IRRADIATION ON THE ABILITY OF PROTOTROPHS TO FERMENT LACTOSE

STRAIN	IRRADIATED INOCULUM			CONTROL INOCULUM		
	POSITIVE	NEGATIVE	%	POSITIVE	NEGATIVE	%
58-161 + Y53	7	18	28%	7	18	28%
Y53 + Y87	0	25	0	0	27	0

Two points are of particular interest. The first is the independent confirmation of Lederberg and Tatum's demonstration of regular recombination in *E. coli*, K12 (see Lederberg⁴). The second is the marked increase in rate of recombination due to the irradiation. The reasons for this increase are unknown, but the fact suggests an interesting adaption, perhaps connected with the abnormal growth pattern of the irradiated cells.

¹ Gates, F. L., "The Reaction of Individual Bacteria to Irradiation with Ultraviolet Light," *Science*, **77**, 350 (1933).

² Robinow, C. F., "Cytological Observations on *Bact. coli*, *Proteus vulgaris* and Various Aerobic Spore-Forming Bacteria with Special Reference to the Nuclear Structures," *J. Hyg. Camb.*, **43**, 413-423 (1944).

³ Dienes, L., *Proc. Soc. Exper. Biol. Med.*, **66**, 314-317 (1947).

⁴ Lederberg, J., "Gene Recombination and Linked Segregations in *Escherichia coli*" *Genetics*, **32**, 505-525 (1947).

STRAIN SPECIFICITY AND PRODUCTION OF ANTIBIOTIC SUBSTANCES. VIII. PRODUCTION OF A GRISEIN-LIKE ANTIBIOTIC BY A STRAIN OF *STREPTOMYCES GRISEUS**

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Since the recent demonstration¹ that certain antibiotic agents produced by actinomycetes possess bacteriostatic and bactericidal properties against *Mycobacterium tuberculosis*, a considerable interest has arisen in a systematic study of similar potentialities among the practically unlimited strains and species of actinomycetes that could be isolated from various natural substrates.^{2, 3}

The present investigations were initiated to determine the presence in the feces of healthy and tuberculous herbivorous animals of actinomycetes which have growth-inhibiting properties against mycobacteria and especially *M. tuberculosis*, and of the production by such organisms of antibiotics which have similar properties.

A culture of an organism belonging to the *Streptomyces* was isolated from the fresh stool of a healthy heifer, and found to be highly effective. This culture (No. 3510) was tested against four mycobacteria, namely, *M. ranae*, *M. avium*, *M. phlei* and *M. tuberculosis* 607, a fast-growing non-pathogenic strain of the human tubercle bacillus. These tests were made by the agar cross-streak method⁴ on three different media: dextrose-asparagine agar, nutrient agar and egg albumin agar. A streptomycin-producing strain of *S. griseus* was included among the organisms for comparison with the more active unknown cultures.

The results presented in table 1 show that the production of active anti-mycobacterial substances differs with the media used for test purposes. The various strains of the mycobacteria also differ in sensitivity to the different actinomycetes. Whereas culture R60 had, for practical purposes, no activity against any of the mycobacteria, A47 had considerable activity on 2 of the mycobacteria on certain media, and HF23 had good activity on all the mycobacteria on nutrient agar, 3510 and the streptomycin-producing strain of *S. griseus* were active against all the mycobacteria tested. There was a difference, however, between the last two, 3510 showing only limited activity on dextrose-asparagine agar. Because of the high level of activity on various substrates against mycobacteria, 3510 was selected for more detailed investigation.

TABLE 1
INHIBITION OF GROWTH OF VARIOUS MYCOBACTERIA BY CERTAIN CULTURES
OF *Streptomyces*

Zone of Inhibition in Mm.*

<i>Streptomyces</i> NO.	<i>M. ranae</i>			<i>M. avium</i>			<i>M. phlei</i>			<i>M. tuberculosis</i> 607		
	DA	NU	EG	DA	NU	EG	DA	NU	EG	DA	NU	EG
R60	0	0	0	0	0	0	±	0	0	0	0	0
A47	0	0	0	0	0	0	±	34	13	0	14	0
HF23	0	15	0	0	15	0	6	36	0	0	20	0
3510	6	20	9	±	19	9	7	25	12	0	32	11
Streptomycin- producing <i>S. griseus</i>	15	27	11	12	28	9	18	25	15	15	27	11

* DA = dextrose-asparagine agar; NU = nutrient agar; EG = egg albumin agar.

On further study, 3510 was found to belong to the group of *S. griseus*. It grew well under both static (surface) and agitated (submerged) conditions and produced an active antibacterial agent. The concentration of the antibiotic depended greatly on the composition of the medium. Lysis of the mycelium appeared after 5 to 7 days in submerged cultures at 28°C., and after 2 weeks or longer in static cultures.

The antibacterial potency of the culture filtrate and of the isolated crude preparation may be determined either by the agar-streak dilution method⁵ or by the agar-diffusion or cup method.⁶ The rapid development of resistance of certain bacterial cells to the antibiotic precludes the general use of serial dilution procedures in liquid media. In general, the degree of resistance observed in using the serial dilution assay techniques is as great as that observed for grisein on some test organisms; however, the capacity of bacterial cells to develop resistance to this antibiotic is selective rather than general, as is the case for grisein. The growth of strains of *E. coli*, for example, in dilutions ranging from 1-4 to 1-2048 incubated for 18 hours at

37°C. cannot be differentiated from the control tube; whereas strain of *S. aureus* under similar conditions will show a clear-cut delineation between tubes containing growth and those not, at varying dilutions depending upon the potency of the culture filtrate or powdered preparation used.

The antibiotic spectra of a typical culture filtrate of 3510, using the agar-streak dilution method, and of a solid preparation obtained from the filtrate were similar, as shown in table 2. The activity was highly selective in nature, including both gram-positive and gram-negative bacteria; fungi were not affected. The culture medium showed occasional activity against *M. tuberculosis* 607; this activity was lost, however, on the isolation of the antibiotic. This points to the possibility that the culture produces a second antibiotic substance which is not removed from the medium or which is inactivated in the purification process.

TABLE 2
ANTIBACTERIAL SPECTRA OF THE CULTURE FILTRATES AND OF SOLID PREPARATION OF 3510

TEST ORGANISM	CULTURE FILTRATE, UNITS/ML.	CRUDE DRY PREPARATION, UNITS/MG.
<i>Escherichia coli</i>	50	12
<i>E. coli</i> , streptomycin-resistant strain	30	20
<i>Serratia marcescens</i>	0	<0.05
<i>Aerobacter aerogenes</i>	0	<0.05
<i>Proteus vulgaris</i>	0	<0.05
<i>Pseudomonas fluorescens</i>	0	<0.05
<i>Shigella dysenteriae</i>	100	40
<i>Sh. paradyenteriae</i>	300	>120
<i>Sh. alcalescens</i>	30	12
<i>Salmonella pullorum</i>	300	>120
<i>Bacillus subtilis</i>	10	4
<i>B. mycoides</i>	0	<0.05
<i>B. circulans</i>	0	<0.05
<i>B. cereus</i>	0	
<i>Staphylococcus aureus</i>	30	>40
<i>Sarcina lutea</i>	0	<0.05
<i>Micrococcus lysodeikticus</i>	0	<0.05
<i>Mycobacterium tuberculosis</i> 607	0-10	<0.05
<i>Cryptococcus neoformans</i>	0	0
<i>Trichophyton mentagrophytes</i>	0	0.4
<i>Candida albicans</i>	0	<0.05

The culture filtrate did not always possess tuberculostatic activity. This substance appeared to be produced in increasing concentrations in the medium, after the activity of the major antibiotic had reached a maximum. This was true especially under submerged conditions of culture and at a temperature of incubation lower than the usual 28°C., namely, at approximately 24°C. All attempts, however, to isolate the tuberculostatic factor

from the metabolic solutions failed. By the use of the cup method and *M. avium* as the test organism with streptomycin as a standard of comparison, 30–50 units/ml. of culture filtrate were commonly obtained. Occasionally as high as 120 units/ml. were observed.

The antibiotic that was isolated from the culture medium had a high activity against gram-negative enteric bacteria, including streptomycin-resistant strains of *E. coli*.

The addition of 50 mg. of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ per liter of nutrient broth yielded culture filtrates and solid preparations with much greater activity than those produced by the best meat extract-corn steep liquor media. When zinc was also added, it was found that there was a critical balance between this element and iron. When 30–35 mg. per liter $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 8–10 mg. per liter $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and 10 g. NaCl per liter were added to peptone-meat extract media in distilled water, good growth and production of the antibiotic were obtained. The addition of 3 g. per liter glucose resulted in even better growth. When this medium was used in static cultures, 2550 *E. coli* units/ml. of culture filtrate were obtained in 4 days at 28°C. The addition of glucose to unbuffered media inhibits the production of the antibiotic, because of the lowering of the pH in the early stages of growth.

When the cell-free culture filtrate was treated with activated charcoal (7–10 g. per liter) all the antibiotic activity was removed from the filtrate. Elution of the adsorbate with neutral 95 per cent ethanol yielded 10–15 per cent of the activity. The eluate was concentrated to dryness *in vacuo*, or ethyl ether was added in a separatory funnel and the aqueous layer collected and concentrated to dryness. When the concentrated liquid was added to acetone, a precipitate was obtained; this was triturated to a powder, washed with ether and desiccated. A yield of 200 to 400 mg. was obtained per liter of medium, depending upon the composition of substrate, rate of growth and antibiotic-producing capacities of the strain used. The preparations thus obtained showed an activity of 12,000 to 20,000 *E. coli* dilution units per gram. The activity against *Shigella paradysenteriae* and *Salmonella pullorum* was nearly 10 times as great.

The various biological and chemical properties of the antibiotic point to its close similarity to grisein, an antibiotic produced by another strain of *S. griseus*.⁷ A comparison of the antibacterial spectra of this antibiotic with certain others produced by actinomycetes is given in table 3.

The similarity of this antibiotic to grisein may be summarized as follows:

1. Both are produced by strains of *S. griseus*.
2. Both are highly selective in their action against gram-positive and gram-negative bacteria, and are especially active against enteric bacteria, antibiotic 3510 having an even narrower antibacterial spectrum than grisein.

TABLE 3
COMPARATIVE ANTIBIOTIC SPECTRA OF STREPTOTHRICIN, STREPTOMYCIN, GRISEIN AND
ANTIBIOTIC 3510

TEST ORGANISM	STREPTO- THRICIN	STREPTO- MYCIN	GRISEIN	ANTIBIOTIC OF 3510
<i>E. coli</i>	+	+	+	+
<i>E. coli</i> *	+	—	+	+
<i>E. coli</i> †	+	+	—	+
<i>Ps. fluorescens</i>	—	+	+	—
<i>S. marcescens</i>	±	+	+	—
<i>A. aerogenes</i>	+	+	—	—
<i>Pr. vulgaris</i>	+	+	—	—
<i>S. aureus</i>	+	+	+	+
<i>B. mycoides</i>	—	+	—	—
<i>B. subtilis</i>	+	+	+	+
<i>B. cereus</i>	—	+	—	—
<i>B. megatherium</i>	+	+	+	—
<i>S. lutea</i>	+	+	±	—
<i>M. lysodeikticus</i>	±	+	+	—

* Streptomycin-resistant.

† Grisein-resistant.

3. Antibiotic 3510 is accompanied occasionally by tuberculostatic activity, a fact which has never been demonstrated under comparable conditions for the grisein-producing strains of *S. griseus*.
4. Antibiotic 3510 is obtained by the same methods of elution from activated carbon as is grisein. All elution methods that failed with grisein have also failed in the isolation of this substance.
5. Antibiotic 3510 and grisein are similar in their solubility in water and insolubility in organic solvents.
6. The two antibiotics are similar in their heat stability.
7. The activity of neither antibiotic is inhibited by cysteine, glucose and horse serum.
8. The production of both antibiotics is favored by the presence of iron in the medium.
9. The activity of both antibiotics is inhibited by certain concentrations of iron in the test medium.
10. Actinophage active against streptomycin-producing strains of *S. griseus* has no activity either against grisein or the 3510 producing strain.
11. Both antibiotics have a greater effect on streptomycin-resistant *E. coli* cells than on the normal non-resistant laboratory strains of this organism.
12. When cross-streaks are made between actinomycetes producing various antibiotics and the same organisms, certain striking differences are obtained, as shown in table 4.

TABLE 4
CROSS-INHIBITION AMONG VARIOUS ANTIBIOTIC-PRODUCING ACTINOMYCETES
Cross-Streak Agar Method, Using Nutrient Agar*

CULTURE NO.	ANTIBIOTIC PRODUCED	TEST ORGANISMS, INHIBITION IN MILLIMETERS			
		3465	3516	3478	3510
3463	Streptomycin	8	29	19	21
3516	Streptothricin VI	9	15	2	3
3478	Grisein 3478	5	22	0	0
3510	Antibiotic 3510	17	25	17	0

* Streaked on agar plate, allowed to grow 48 hours at 28°C., then cross-streaked by test cultures.

13. Streptomycin-resistant *E. coli* cultures as well as grisein-resistant cultures are sensitive to antibiotic 3510.
14. *S. griseus* 3510 is active against the Bodenheimer bacterium, which is resistant to both grisein- and streptomycin-producing strains of *S. griseus*.

Except for certain minor differences, antibiotic 3510 is most similar to grisein. Until this antibiotic has been isolated in a pure form and its chemical composition established, it shall be designated as antibiotic 3510 and may be considered as a grisein-like substance.

When tested against several common bacteria by the use of the cup method, streptomycin was found to give the smallest zones of inhibition against *E. coli*, larger zones against *S. aureus* and the largest zones against *B. subtilis*. Grisein produces the smallest zones with *S. aureus*, larger zones with *B. subtilis* and the largest zones with *E. coli*. Antibiotic 3510 produces smallest zones with *B. subtilis*, larger zones with *E. coli* and the largest zones with *S. aureus*.

Antibiotic 3510 was found to have marked *in vivo* activity. This was established by injecting yolk sacs of 9-day-old chick embryos previously infected by the same route with a suspension of *Salmonella pullorum*. For this purpose crude preparations of the substance assaying by the cup method 45 streptomycin units per milligram were used. Chick embryos with 0.1 ml. of a 10^8 dilution of a 24-hour heart broth culture representing about 150 cells thus infected die regularly in 18 hours or less.

All the uninfected controls, both untreated and treated, survived, as did the controls injected with sterile physiological saline. All infected controls died in 24 hours. When streptomycin was used, all the infected eggs with μ g. died in 24 hours; 1000 μ g. allowed 3 out of 5 infected eggs to survive for 24 hours and beyond. The infected eggs treated with 10 units of antibiotic 3510 died in 24 hours; with 100 units, 1 out of 5 survived 24 hours and throughout the experiment; 500 units allowed all the eggs to survive 24 hours, and 4 out of 5 survived from 48 hours through 15 days, at which time the experiment was concluded (table 5).

TABLE 5
COMPARATIVE EFFECT OF STREPTOMYCIN AND ANTIBIOTIC 3510 UPON *Salmonella pullorum* IN CHICK EMBRYOS

NO. OF EMBRYOS TREATED	TREATMENT*	AMOUNT OF ANTIBIOTIC PER EMBRYO, UNITS	NO. OF EMBRYOS SURVIVING, DAYS		
			1	2	15
5	Uninfected	..	5	5	5
5	Infected, untreated	..	0	0	0
5	Streptomycin	100	0	0	0
5	Streptomycin	500	0	0	0
5	Streptomycin	1000	3	3	3
5	Antibiotic 3510	10	0	0	0
5	Antibiotic 3510	100	1	1	1
5	Antibiotic 3510	500	5	4	4

* All embryos tolerated well 1000 streptomycin units or 500 units of antibiotic 3510.

In view of the relatively low toxicity of crude preparations of antibiotic 3510, high solubility in water, relative stability, lack of inhibition by serum, activity *in vivo*, high level of activity on gram-negative enteric pathogens and activity upon streptomycin- and grisein-resistant bacteria, this antibiotic offers certain possibilities for the control of infections caused by enteric pathogens, especially those resistant to other antibiotics.

Summary.—A grisein-like antibiotic, designated as antibiotic 3510, was found to be produced by a strain of *S. griseus* isolated from the intestinal contents of a heifer.

Antibiotic 3510 has a very narrow antibacterial spectrum, even narrower than that of grisein. It is active against certain gram-positive and gram-negative bacteria, especially organisms of enteric origin. Bacteria that have been made resistant by serial passage to streptomycin and to grisein are still sensitive to this grisein-like substance.

Antibiotic 3510 shows, in crude preparations, a rather low toxicity for the chick embryo. It is capable of protecting the latter against infections with *Salmonella pullorum*. In equal concentrations, it appears to be more potent than streptomycin.

The strain of *S. griseus* which produces the grisein-like antibiotic 3510 forms another antibiotic which inhibits the growth of certain mycobacteria, including *M. avium* and *M. tuberculosis* 607.⁸

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‡ The authors are indebted to Dr. J. D. Thayer, of the U. S. Public Health Service V. D. Research Laboratories, for his aid in testing the toxicity and *in vivo* effectiveness of antibiotic 3510.

THE GAS TURBINE AND ITS SIGNIFICANCE AS A PRIME MOVER

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Introduction.—The emergence of the gas turbine as an accepted member of the family of heat engines and prime movers has taken place within the last ten years. Its application to the field of aircraft propulsion, notably in the form of jet propulsion, has been accompanied by much publicity and fanfare. This application, while enormously important, has tended to obscure the wider implications of this development. This paper gives a brief survey of developments which led to the gas turbine and their implications for the future.

In the two centuries and a half during which man has occupied himself with heat engines, there have been only a few events of far-reaching implications, and only three major types of prime movers have reached maturity: the steam engine, the steam turbine and the internal combustion engine. The selection from technological history is somewhat arbitrary, but up to the end of the last century the following events and approximate dates may be singled out as of particular significance: Savary's water raising engine, 1700; Newcomen's atmospheric engine, 1710; Watt's condensing engine, 1770; the caloric engine (Cayley, Stirling, etc.), 1825; Otto's internal combustion engine, 1875; the steam turbine (Parsons and de Laval), 1885; the Diesel engine, 1895. The arrival of the gas turbine is thus an event of the first importance. If it had taken place in a less exciting period of history, it would have been regarded as truly epoch making. Competing as it does with atomic bombs, atomic power, supersonic airplanes and guided missiles, not to mention the political and cultural characteristics of a "time of troubles," its relative significance has been overshadowed by other events.

Survey of Historical Background.—The gas turbine represents the fulfillment of an idea which was born at the beginning of the last century with

Carnot's theory of the motive power of heat. Roughly, the idea is to compress a working gas, such as air, impart heat to it at as high a temperature as possible, and then expand it back to the original pressure. The excess of work released in the expansion, over that required by the compression, is the useful output. Carnot showed that its relation to the energy supplied in heating the gas depends only upon the upper and lower limits of temperature. This is the basic principle of all heat engines. The steam engine of Carnot's time proved to be a poor approximation of this idea, however, because its functioning was tied to the peculiar properties of water vapor.

The *hot air engine*, or the *caloric engine*, was the first direct embodiment of Carnot's idea. During the first half of the last century, many of the distinguished leaders of the mechanical engineering profession devoted themselves to the development of this engine. In this process practically all of the major inventions relating to heat cycles, such as regeneration, combustion of fuel under pressure, and so on, were made and applied, only to be forgotten and reinvented later in other contexts. Many such engines were indeed built, and some lingered in certain applications until the end of the century. With the state of the art of mechanical engineering and metallurgy at the time, the full realization of the idea was impossible. The caloric engine represents an interesting example of a stillborn development whose basic logic was far in advance of its means of realization.

This failure left a deep impression on the profession, and the impracticability of this type of cycle was thenceforth taken for granted. Under the heading "Air Engines," the fourteenth edition (1932) of the *Encyclopedia Britannica* expresses the situation as follows: "The practical drawbacks to employing air as the working substance of a heat engine are so great that its use has been very limited. Such attempts as have been made to design air engines on a large scale have been practical failures, and are now interesting only as steps in the historical development of thermodynamics."*

During the succeeding century, the attention was shifted to improvements in the steam engine and the steam cycle as a whole. With the arrival of the steam turbine and the resulting possibilities for advance of pressures and temperatures, this reached a high state of perfection, and it is still the major type of power plant in the field of large units. It is significant, however, that it was not until after World War I that the refinements of regeneration and reheating came into general application.

It was in the internal combustion engine that the dream of an ideal power cycle first found an embodiment which reached full maturity. This is the ideal reciprocating type of air engine, representing a clever compromise, which avoided many of the difficulties which had been encountered in the caloric engine. With its application to transportation, the incentive for a successful internal combustion engine was so great that its development became a major preoccupation of industry.

By the end of World War I the limitations of the internal combustion engine had become apparent. These were to influence profoundly power developments of the following two decades. The method of *supercharging* was introduced by *Rateau* and others in the early part of this century to overcome the limitation in inlet volume. It provided the incentive for the development of efficient rotary compressors. As in all reciprocating engines, the gas cannot be expanded to a pressure lower than that corresponding to the initial volume. Since the gas is much hotter at the exhaust than at the inlet, this means that the exhaust pressure is above the atmospheric pressure, causing a considerable portion of the available energy to be lost. Some of it can be recovered if the supercharger compressor is driven by a turbine running in the exhaust gas stream. This idea was introduced by *Buchi* in the nineteen twenties. This *turbine-driven supercharger* compares, in relative significance, with the major prime mover developments. It is of particular value in connection with aircraft power plants, where it was introduced by *Moss* and others.

The idea of the perfect air engine never died completely, however, and after the introduction of the steam turbine, it reappeared in the device which we now know as the gas turbine and which utilizes the same processes previously tried in the caloric engines, but now without the limitations of reciprocating machinery. Many gas turbines were built in the years before and after the turn of the century, but all of them were failures, and many of them did not even run by their own power. Part of this was due to the limitations in the state of the art of mechanical engineering, but it was also because the full implications of Carnot's idea were not sufficiently realized, particularly the fact that the excess power delivered in the expansion over the power consumed by the compression is a very sensitive function of the temperature and the efficiency of the elements. Part of these efforts were applied to schemes involving combustion at constant volume along the lines of the internal combustion engine. While this development by *Holzwarth* did not lead to a commercial prime mover, it provided much essential experience in high-temperature turbine construction.

In the nineteen thirties some of these efforts led to commercial devices. In Switzerland, where most of this early gas turbine history was made, successful gas turbines were built before the outbreak of World War II. The *Velox* steam boiler had a combustion circuit, which to all intents and purposes functioned as a gas turbine, even if it was merely an auxiliary. Applications to the *Houdry* cracking process followed. Some of these units were also built and installed in the United States. A small reserve power plant and a locomotive were in operation at the outbreak of the war in 1939. All of these plants made use of relatively low gas temperatures with resulting low efficiencies.

The application of the turbine-driven supercharger to aircraft power

plants proved to be essential to flight at high altitudes. Under this incentive remarkable progress was made in the United States during the nineteen thirties in high-speed compressors and in the development of alloys with the required mechanical strength and resistance to corrosion at high temperatures. Some progress in the development of such materials had already been made in other branches of industry. The two principal premises for a successful gas turbine (an efficient compressor and an efficient, high-temperature turbine) thus came together for the first time in the turbine-driven supercharger for airplanes. As soon as World War II was under way, we realized with a start that, as part of the war production, we were producing successful gas turbines by the thousands.

Some developments of gas turbines for general prime mover purposes were carried out during the war, but it was the aircraft power plant itself that was destined to be the first and major beneficiary, because of the possibility of *jet propulsion*. To produce propulsive force by the ejection of matter is an old principle of locomotion, also used by many marine animals. To be effective from the point of view of economy of energy it is required that large amounts of matter be ejected at velocities of the same order of magnitude as the speed of locomotion. These premises were fulfilled for the first time in man's history in the combination of a gas turbine and an airplane flying at speeds in excess of 500 miles per hour.

A preliminary evaluation of jet propulsion of planes was made in the United States in 1922, but the premises were then not favorable. Application of jet propulsion to planes seems to have been made first in Germany, then in England, and last in the United States. By 1941 serious efforts were under way in all three countries, and towards the end of the war jet propelled planes were flown by the air forces of the three nations. The *turbo jet* is now a major type of aircraft power plant. The *turbo prop*, in which the gas turbine drives a propeller, has lagged behind in its development, principally because its need has not been quite so urgent.

This view in retrospect of the history of the heat engine is interesting and instructive in appraising the future of other ideas. After man had succeeded in lifting weights by means of heat energy through steam power, it required more than a century for the basic principles to be formulated. The type of power cycle which followed by logical deductions from these principles required another century for its realization. Specifically, man had to develop the steam engine, the internal combustion engine, the steam turbine, the Diesel engine and the airplane, with their attendant background of technology, before he was ready to tackle with success the simple ideas inherent in the original caloric engine.

The Future of the Gas Turbine.—The successful prime mover represents a compromise between a great variety of conflicting considerations, of which the thermodynamic efficiency is only one and not always the most im-

portant. Reliability, cost, weight, space, maneuvering ability, ease of manufacturing and maintenance and fuel requirements are examples. The situation is exemplified by such extremes as the modern aircraft engine, weighing less than a pound per horsepower and requiring the utmost in manufacturing precision and fuel specifications, as contrasted with a modern steam plant, weighing perhaps 200 pounds per horsepower but requiring only moderately complicated manufacturing facilities and capable of burning the lowest grades of coal. Both of these solutions represent the designer's interpretation of the optimum compromise of all factors. Neither can be regarded as superior to the other, although it is certain that both could benefit from exchanges of the two points of view.

The gas turbine represents a compromise between the steam power plant and the internal combustion engine. It is hoped that it will have the superior qualities of maintenance possessed by the former and the efficiency characteristics of the latter. Up to the present time the successful gas turbines have operated on Diesel fuel, but there is promise of satisfactory performance on lower grades of oil. Experiments are now under way to burn coal directly in at least one locomotive application. If this should eventually become successful, the gas turbine would have gained an enormous potential field of application.

One of the most important characteristics of the gas turbine, in comparison with both the internal combustion engine and the steam power plant, is that it does not normally depend upon large quantities of cooling water. This may become of great importance in districts such as the American Southwest and will always weigh heavily in locomotive applications.

In its present form, the gas turbine is not easily made reversible, which is one of its major drawbacks. Applications to ships and locomotives are thus planned for electric drive, while some marine applications are planned with adjustable pitch propellers.

Active developments of gas turbines for stationary power plants, locomotives and ships are under way in many countries. Switzerland is still leading in these fields, followed by England and the United States. The total construction is still relatively small in terms of general power developments, but the pace is significant in comparison with earlier prime mover developments. According to an estimate made recently by the Gas Turbine Division of the American Society of Mechanical Engineers, the total capacity of gas turbines, including those under construction, is twenty-seven plants, totaling about 200,000 kw. for stationary power applications, and twenty-seven plants, totaling about 100,000 hp. for locomotives and ships. Perhaps the most significant is the American development of two coal burning locomotives, which, if successful, will have great influence upon future locomotive construction. •

It is not expected that the gas turbine will invade the automotive field,

at least in the near future. It is not easily adapted to power plants of small capacity, although successful developments in this direction are not wholly improbable. Should such a development take place, it would have a far-reaching influence upon the business of refining crude oil. Gasoline is not a particularly suitable fuel for gas turbines, and most of the refinements peculiar to the internal combustion engine would cease to have any value. The air forces are already confronted with this problem.

The discovery of atomic power is often considered to have the most far-reaching effects of all upon the prime mover field. So far, however, atomic energy is merely another fuel, the use of which is subject to the same limitations in its conversion to power as any other source of heat. No facts have yet been revealed which would suggest a change in this situation, and there are at present many sources of low-grade energy which could compete commercially with atomic power. Rapid progress is certain in this field, however. No definite information is available as yet to indicate whether the steam power plant or the gas turbine is preferable for the utilization of atomic heat. Both may be used.

So far, the gas turbine has completely revolutionized only one field, that of aircraft propulsion. Here the potentialities of the new prime mover have already been seized upon by the airplane designers, and the major undertakings on the border of capacity and speed are already wholly outside the range of the internal combustion engine. The disappearance of the internal combustion engine for large powers at high speeds is therefore only a matter of time.

To avoid misunderstanding, it is necessary to point out that, at the moment, the internal combustion airplane engine alone has been developed to the adequate state of reliability, and so far it is the only type of engine which can be quickly produced in large quantities. For some time, therefore, it will be necessary to retain the internal combustion engine, particularly for long-range applications.

As the development can be visualized at the present time, the aircraft power plant will exist in the following versions, depending upon the flight speed. For the lowest speeds, the *piston engine*, driving a propeller, will remain. Beyond this range and up to about 500 miles per hour, the *turbo prop* appears to be the most logical for all long-range aircraft. Beyond this and into the supersonic range, the *turbo jet* has its best field of application, with the modification known as the *ducted fan* preferable for long range. The *ram jet* will have its field of application for the extremes in speed probable only in missiles.

When it is considered that each one of these four versions of gas turbine applications will require systematic programs of applied research in fluid mechanics, thermodynamics, mechanics and metallurgy, and the solution of wholly new manufacturing, production and testing problems, the impli-

cations of the new developments are staggering and beyond immediate comprehension. But the social implications are even greater. Mr. Churchill in *The Second World War* refers to "the unlucky discovery by an immature civilization of the internal combustion engine and the art of flying." The internal combustion engine was but an intermediate step. The implications of the new prime mover when fully developed, if used with equal lack of judgment, will make the mischief done by the internal combustion engine seem like the pranks of immature boys.

* It is a curious fact that a hot air engine of nearly the original Stirling design has recently been brought out in Holland with impressive claims for its merits. Its real significance is yet to be appraised.

NEW FACTS ON SEX DETERMINATION IN *DROSOPHILA MELANOGASTER*

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According to the text books sex determination in *Drosophila* is completely understood: a series of female determining factors in the X-chromosome are balanced against a series of male determiners spread all over the autosomes. Leaving aside the problem of the female determiners, it must be stated that thus far neither a single autosomal male factor nor a group of them has been discovered although their existence was assumed on the basis of the occurrence of triploid intersexes of the constitution 3A2X (see discussion below). In the course of work on heterochromatic heredity (to be presented in detail soon) facts were found which lead a considerable step forward toward the understanding of sex determination in *Drosophila*.

In the work mentioned (the analysis of the podoptera effect) it was found that the dominant mutant Beaded (Bd 3—93.8) is probably a mutant of a heterochromatic section (as opposed to the ordinary euchromatic mutants) and the same conclusion was drawn for the Minutes. In the course of the analysis Beaded was combined with a third chromosome Minute (M(3)w). It turned out that all flies containing both dominants in heterozygous condition (both are homozygous lethals) showed 100% dominance of Bd instead of the 5% found approximately in Bd/+; furthermore, a considerable percentage of the males were intersexual. Subsequently Bd was combined with a number of Minutes in all autosomes. The results are summarized in table 1. Only in No. 11 with M(2)l 2 and No. 14 with M-4 were

TABLE 1
F₁ ♂ Bd AND M

COMBINATION	GRN.	MOTHER	♂ %				
			+	LOW INT.	MED. INT.	HIGH INT.	EXTL. INT.
1. Bd/M(3)36c	F ₁	Bd	100? a few?				..
2. Bd/M(3)w	F ₁	Bd	51		49		..
3. Bd/M(3)w	F ₁	M	33	25	34	8	..
4. Bd/M(3)w	F ₁	Bd XXY	54	11	22	13	..
5. Bd/M(3)S34	F ₁	Bd	..	5	..	95	..
6. Bd/M(3)B ³	F ₁	Bd	62	38			..
7. Bd/M(3)S37	F ₁	Bd	9	29	29	33	..
8. Bd/+ M(2)l/+ (a px Df)	F ₁	Bd	..	9	58	33	..
9. Bd/+ M(2)173/+	F ₁	Bd	74	11	13	2	..
10. Bd/+ M(2)S7/+	F ₁	Bd	48	14	20	18	..
11. Bd/+ M(2)l2/+	F ₁	Bd	100				..
12. Bd/+ M(2)S13/+	F ₁	Bd	82	..	6	12	..
13. Bd/+ M(2)B/+	F ₁	Bd	97	1.5	1.5		..
14. Bd/+ M(4)/+	F ₁	Bd	100				..

all males with both dominants normal (though the Bd dominance effect was the same, namely, 100% dominance in all cases). All other Bd M combinations produced intersexual males. In all these F₁ crosses the segregating Bd/In, M/In, etc., served as controls (Bd and M lines are balanced over inversions). The intersexes all had the sex comb or rudiments of it. They were classified for the genital armature which varied from the absence of the penis to almost female structure. In the highest grades of intersexuality found the abdomen was also more or less female in shape, and preliminary dissections left no doubt that male intersexuality was involved. In some combinations, e.g., Nos. 1, 12 and 13 only a small percentage of the males were intersexual. In others, about one half of the males were intersexual (Nos. 2, 3, 4, 6, 10). Once (No. 7) the majority of the males were intersexual, and twice (Nos. 5, 8) all males were intersexual and all exhibited the high classes of intersexuality. All these results were constant in repeated crosses. It should be added that the higher the average degree of intersexuality the lower the viability of the Bd M males as compared with the controls. In the most extreme cases (Nos. 5, 8) the number of Bd M males was very small in the individual one pair crosses, and they emerged on the 18-22d day after all other flies had hatched. As the table shows, there is no basic difference between second and third chromosome Minutes. In combinations with either group all degrees of variation in the percentage and mean degree of intersexuality were represented.

The important question is the necessity of Bd for the effect observed. Combinations of a considerable number of Minutes were made so that one male carried two different Minutes. Among thousands of normal males a

few (4) flies were found with abnormal genitals, and these differed from typical intersexes. Thus it is almost certain that Bd is needed for the production of intersexuality. Further experiments are planned to confirm these findings. A series of dissections of the abnormal males will be needed to establish their exact nature.

If Bd M pushes the male determination in the female direction, this must mean that the action of the male determiners has been weakened. If this is the case, females containing Bd M should be more female, i.e., incipient hyperfemales. Actually in the most extreme combinations (Nos. 5, 8) the females regularly showed abnormalities of the abdominal tip, as well as frequent absence or different abnormalities of the anal plate. Although these features do not resemble those of triplo-*X* hyperfemales, I consider them as signs of hyperfemaleness, pending a more detailed anatomical study.

It was assumed as a working hypothesis that Bd has the decisive action but that the addition of a Minute is required to push the developmental system over the threshold of the normal sex balance. (Many other dominants were combined with Bd with negative results.) If this is true, a second Minute might still increase the degree of intersexuality as a kind of dosage effect. Therefore, a number of combinations of Bd with both a second and third chromosome Minute were made using the standard balanced stocks. This requires crossing Bd with a second chromosome Minute, and crossing extracted Bd M phenotypes with a third chromosome Minute. Three classes of flies, simultaneously Bd and M, are thus produced only one of which is Bd M(2) M(3). This class cannot be distinguished in these crosses. (The experiments will be repeated after appropriate markers have been introduced.) Thus the results assembled in table 2

TABLE 2
♂ Bd PLUS TWO MINUTES IN SECOND AND THIRD CHROMOSOMES

COMBINATION	MOTHER	+	LOW	MED.	HIGH	EXTR. INT.
Bd-M(3)w-M(2)S13	M(3)w	34	8	16	34	8
Bd-M(3)w-M(2)S13	Bd/+ M(2)213/+	51	6	10	23	10
Bd-M(3)w-M(2)l2	Bd/+ M(2)l2/+	56	8	20	16	..
Bd-M(3)w-M(2)B	Bd/+ M(2)B/+	67	33	..
Bd-M(3)w-M(2)l	Bd M(3)w/+
(a) Bd M(3)w	33	67	..
(b) Bd M(3w)/+ M(2)l/+	11	56	33

for Bd M males are those for all 3 combinations together, i.e., Bd M(2) M(3), BdM (3) and Bd M(2). The positive result, i.e., further shifting toward femaleness in Bd M M can be seen in the fact that not only are the high-grade intersexes preponderant, but that a still higher class, not recorded in *F*₁, appears in considerable numbers. There is one cross recorded in this table (the last one) which permits comparison of Bd M with

Bd M M flies because the mother was a crossover fly with Bd and M(3)w in the same chromosome. This female was crossed to M(2)l/Cy. The Bd M Cy flies are therefore Bd M(3)w/+ and the Bd M not Cy flies are Bd M(3)w/+; M(2)l/+. The result (*a* and *b*) shows convincingly a shift toward more intersexuality in the presence of both Minutes.

When F_2 and backcrosses were made involving Bd and M(3)w, it was first noticed that the Bd effect upon the wings was greatly increased in some combinations but not in others. This is an unexpected result since recombinations of modifiers should have been identical for the crosses. In following up this result it was realized that the percentage and grade of intersexual males differed according to the genetic constitution of the mother. If the mother of a backcross was Bd or M, the result was similar to that obtained in F_1 . If the mother was Bd/M or Bd M/+, the grade and percentage of intersexuality were considerably increased regardless of whether the father was Bd, M, M/Bd or wild type, the latter mating with a crossover mother. Table 3 contains the relevant data. A glance over the high

TABLE 3
R F_2 , ETC., Bd AND M WITH DIFFERENT MOTHERS

CROSS	MOTHER	% ♂ Bd M				
		+	LOW	MRD.	HIGH	EXTRE. INT.
Bd/M(3w) × Bd	Bd/M(3)w	4	96	..
Crossover Bd M(3)w/+ × Flor	Bd M(3)w/+	9	..	0	82	..
Bd/M(3)w × Bd	Bd/M(3)w	5	95	..
Bd/M(3)w × Bd/M(3)w	Bd/M(3)w	4	14	32	50	..
Bd/M(3)w × M(3)w	Bd/M(3)w	27	..	6	67	..
Bd/M(3)w × Bd	Bd/M(3)w	..	8	30	50	12
M(3)w × Bd/M(3)w	M(3)w	31	..	69
M(3)w × Bd/M(3)w	M(3)w	32	14	40	14	..
M(3)w × Bd/M(3)w	M(3)w	24	17	43	16	..
All F_1 , R F_1 , etc., Bd/M(3)w	Bd or M	42	17	27	14	.. (n = 425)
All F_1 , R F_1 , etc., Bd/M(3)w	Bd/M	6	7	17	68	12 (n = 203)

intersexuality classes shows clearly the result and a summary of all crosses of both types in the last two lines is very convincing. These facts can mean only that the genetic constitution of the egg before the maturation divisions is of importance, i.e., that the action of the male determiners occurs at least in part in the growing egg by conditioning its cytoplasm, in this case toward weakened maleness. This result was thus far obtained only for this one combination (Bd and M(3)w) and will have to be checked for the other Minutes.

Discussion.—The first problem is that of interpreting these results genetically. Is the +^{Bd} section of the third chromosome a male sex determiner which, if changed by mutation to Bd, is weakened in its male determining function? Are all the +^M loci in the second and third chromo-

somes male sex determiners which are again weakened by the M mutant? Is the weakening of all these loci individually not enough to push the sex balance below the male threshold; and is it strong enough only at the Bd locus so as to allow a small additional push (the added M) to shift it below the threshold? The experiments, as far as they go at present, do not permit a decisive answer. But the fact that male sex determiners have been confined to the third chromosome by Mrs. Pipkin (see below) and the recent work of Sturtevant (see below) favor the assumption that the +^{Bd} region of the third chromosome acts as a major male determiner. If this is the case, a Bd allele might be found which acts exactly as the Bd M combination does in our case. (It is remarkable that the only Bd allele which I could test thus far, namely Bd^G, most extreme in wing effect and pene- trance, does not show any sex effect in combination with the Minutes. One might think that Bd^G changes the locus in the opposite direction from Bd.)

The Minutes are characterized by acting more or less alike in regard to phenotypic effect and lethality. Many of them are visible deficiencies and the rest may be deficiencies also, but on a submicroscopic level. Many of them prolong development at a certain larval stage. Thus we may assume that their action upon development is generalized and that they should not be thought of as mutants of a male determining chromosome section. It is possible that their collaboration with Bd is based upon their effect on the speed of development. The pronounced effect of the M(2)l deficiency is a point in favor of this assumption and will be tested in subsequent work. As the yet unpublished work on podoptera points to a heterochromatic nature of both Bd and M sections, my present working hypothesis is that the so-called male determiners in *D. melanogaster* are identical with an intercalary section of heterochromatin around the Bd locus; further, that the action of this section requires its presence in entirety; that a change in it (deficiency?) weakens its male determining action; that other heterochromatic deficiencies can change the entire developmental system, i.e., the properly balanced speeds of the integral reactions so as to lower the male action below the normal threshold. If these assumptions should turn out by further work to be correct, it would follow that what have been called female determining genes in the X-chromosome will prove to be some of the intercalary heterochromatic sections of the chromosome. I have already tried to produce effects upon the female sex paralleling those reported here for the male sex, but, thus far, without success.

Returning once more to the introduction of this paper, I should like to draw attention to the following. Since Bridges found triploid intersexuality (which had been discovered by Standfuss in moths), it has become customary among *Drosophila* workers to say that sex here is determined by the balance between the determiners in the X and those spread over the autosomes; the reason being that the use of the triploid technique demonstrates

only the total effect of all autosomes. But it has been shown subsequently that the fourth chromosome does not contain male determiners, and recent work of Sarah Bedichek Pipkin (1947) has completely ruled out the second chromosome for *D. melanogaster* as far as sex determining action is concerned. I wonder whether the future will not reveal Sturtevant's dominant strong male factor of *D. neorepleta* (see below) and the section involved in this study of the third chromosome in *D. melanogaster* as the male determiners of *Drosophila*.

A second part of this discussion is prompted by the reawakening of interest in the rôle of the cytoplasm in heredity and the relation between genic action of the chromosomes and the cytoplasmic substratum, including also the visible and biochemical exchanges between nucleus and cytoplasm. It is not my intention to review the old literature in these fields. The genetical aspect, as it appeared before the recent discoveries in lower organisms, has been discussed in my "Physiological Genetics." The Cytological and cytochemical facts connected with the terms chromidia, trophochromatin, etc., prior to the recent work of Brachet, Caspersson, and others, are found in Wilson's "The Cell." The point to be emphasized here touches essentially still another relationship, namely, that between nuclear determinants, cytoplasm and embryological determination. One aspect of this problem is involved in the phenomenon of maternal inheritance which was discovered for certain colors of the silkworm egg by Toyama (1909) (see also Tanaka, 1924). Maternal inheritance is Mendelian inheritance of an egg-character determined prior to fertilization and resulting in an apparent shift of Mendelian behavior by one generation and maternal behavior in what appears as F_1 . The best analyzed cases are Boycott and Diver's analysis of right-left-handedness in snails (1930) (the correct interpretation was first suggested by Sturtevant, 1920) and the analysis of hereditary gynandromorphism via double fertilization in the silkworm, by Goldschmidt and Katsuki (1927 ff.). Both egg color and gynandromorphism in the silkworm are clear-cut effects of a recessive mutant upon an egg character, i.e., pigment formation in one, and specific behavior of a polar nucleus in the other instance. But some of Toyama's studies indicate that the effect of the mutant locus upon a cytoplasmic chemical process is continued beyond fertilization into early development. In the case of the snails the maternal influence is still more prolonged. The mutant here affects a molecular pattern of the cytoplasm, visible in the arrangement of the division spindle. It is possible that the dextrality or sinistrality of the subsequent development affecting the entire body is nothing but a mechanical consequence of the arrangement of the first blastomeres. But it is also possible that the genetically controlled molecular pattern of the protoplasm continues and actively influences the developmental pattern. I am not aware of any information deciding these alternatives.

The most extreme situation of such a nature has been found in the realm of sex determination. I showed (1912-1920) that, in the gypsy moth, sex (or intersexuality) is determined by the balance between male determiners in the *X*-chromosomes and female determiners inherited purely maternally. Because cases were found and analyzed experimentally which could not be explained except by non-disjunction of the sex chromosomes, it was concluded that the female determiner was located in the *Y*-chromosome. As the female is here the heterogametic sex, males never have a *Y*-chromosome although each egg contains a *Y* before meiosis. But all grades of male intersexes are produced in specific crosses, and since this involves production of female features in all organs including those of very late differentiation, the potency for the reaction determining femaleness and even its specific dosage must be present throughout development. Therefore, the *Y*-chromosomal factor must have conditioned the egg cytoplasm before fertilization for such subsequent action as is revealed in the female determining processes of development and their share in the sex balance. When I first discussed these consequences (1920), I realized that, at that time, it was difficult to accept such a conclusion.* Today, after the recent work on infusoria, there are no mental difficulties left.

Our old conclusions, based thus far only on *Lymantria*, which seemed to be a unique case, have now found unexpected support since Sturtevant (1946) found facts in *Drosophila* which parallel completely, *mutatis mutandis*, those in *Lymantria* both as regards the workings of the sexual balance and the rôle of the cytoplasm. (1) Intersexuality is produced by crosses between geographically different oecospecies (in *Lymantria* distant geographic races). (2) Female intersexuality is the result of an unbalance between a strong male determiner from *D. neorepleta* with two weak female determining *X*-chromosomes from *D. repleta*. (In *Lymantria* the male is homogametic, therefore all sexes are the opposite of *Drosophila*, i.e., the same result applies to intersexual males in *Lymantria*.) *D. neorepleta* and *D. repleta* thus behave like strong and weak races in *Lymantria*. (3) While in *Lymantria* the non *X*-chromosomal factor is located in the *Y*-chromosome, it is an autosomal dominant in these *Drosophila* species because the *Y* is here inert. Nevertheless, the autosomal dominant acts, just as the *Y* in *Lymantria*, upon the egg before fertilization. Here actually the autosomal factor has a chance to act later in development as it is present in all female eggs (as opposed to the *Y* in *Lymantria*), but the experiments show the remarkable fact that it does not do so, but acts only before fertilization just as the *Y* in *Lymantria*. Now we know from the facts of gynandromorphism that *X*-chromosomal sex factors can act at any point of the life cycle. This shows the *Lymantria* type of balance (namely, balance between the *X*-chromosomal factors and those outside of the *X*-chromosomes, *Y* or autosomal, which again requires one of the sex-determining reactions, that

corresponding to the heterogametic sex, to occur before fertilization, i.e., via the egg protoplasm) to be a widespread if not a universal type.

Thus it is especially remarkable that we find the same situation again in *D. melanogaster* (table 3). The importance of such facts for an understanding of the respective rôle of nucleus and cytoplasm in development is obvious.

* Actually I tried later to show that cytoplasmic inheritance might be preferable to that in the *Y*-chromosome. I have since shown (1942) why the original analysis must stand.

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PIGMENTS OF YELLOW-EYED RACES OF THE BLACK-EYED SUSAN (*RUDBECKIA HIERTA*)*

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In 1921 one¹ of us reported on the breeding behavior of two genetically distinct types of yellow-coned plants in the Black-eyed Susan which normally has cones with purple florets. It was shown that the two types, though phenotypically alike, could be separated into Black Yellows and Red Yellows by the fact that the cones of the former turn black when treated with alkali and those of the latter turn red when similarly treated. The first of our Black Yellow plants came from open pollinated seed from a purple-coned plant collected from the wild in the neighborhood of Storrs, Conn., in 1910. No Black Yellow plant had been found in nature since then until this past summer when a single yellow-coned plant was discovered at Saratoga Springs which by later test proved to be a Black Yellow. Red Yellows had been earlier found in two localities near Storrs. In one, about a mile from the University of Connecticut, only a single plant was observed. In the other locality, 6 or 7 miles away, several Red Yellows were found within an area of not over an acre. Since these are the only wild yellow-

coned plants observed by the second author who for over 35 years has been interested in looking at fields of *Rudbeckia*, it can be concluded that yellow-coned types are extremely rare in nature.

So far as we are aware the present case was the first in which a sharp chemical distinction could be established between the genetic groups within a phenotype, although, theoretically, all complementary types should differ chemically. The discovery of a reagent which would distinguish the two genetic types was made by testing out a considerable number of chemicals at random without any knowledge of the chemical process that might be involved. It is now possible to say something as to the chemical basis of the tests which were established empirically about 30 years ago.

The chemical tests reported in the present preliminary paper were made by the first author (S.) with florets of the Black Yellow plant found in Saratoga Springs and with those of Red Yellow plants which had segregated out of our horticultural types of tetraploid Black-eyed Susans.

The sap-soluble pigments of plants fall into two main groups (a) anthoxanthins (flavones and flavonols, yellow or cream in color) and (b) anthocyanins which are red, purple or blue. The two groups are easily separated, since the anthoxanthins are soluble, anthocyanins insoluble in Ethyl Acetate. The crude pigments are extracted from the flowers by grinding in dilute (1 per cent) HCl and filtering. The clear filtrate is then shaken repeatedly with Ethyl Acetate, and thereby separated into two fractions, an E. A. fraction containing any anthoxanthins present, and an aqueous fraction which contains anthocyanins or leuco-anthocyanins (i.e., colorless anthocyanin precursors). The two fractions may then be subjected to various colorimetric tests which have been summarized by Scott-Moncrieff.²

Black Yellow Type from Saratoga Springs.—With conc. (8N) KOH gave black color (Black Yellow type). A portion of untreated cone was extracted with 1 per cent HCl and the filtrate exhaustively extracted with Ethyl Acetate. (Three successive shakings with equal bulk of Ethyl Acetate.)

(a) *Ethyl Acetate Fraction.*—On evaporation to dryness on a water bath, a very small residue, creamy white in color, was obtained which when taken up in a small quantity of alcohol gave an almost colorless solution. On addition of a few drops of 1 per cent KOH a deep golden color was produced showing that an anthoxanthin was present.

(b) *Aqueous Fraction.*—This was quite colorless and gave no color reaction with dilute KOH. On boiling, with conc. HCl however, a deep purple red color was developed, showing that an anthocyanin precursor (leuco-anthocyanin) was present.

Red Yellow Type (Tetraploid) from Garden Culture.—With conc. (8N) KOH gave red color (Red Yellow type).

(a) *Ethyl Acetate Fraction.*—This contained an anthoxanthin and in

rather larger quantities than in the corresponding fraction from the Black Yellow type.

(b) *Aqueous Fraction*.—This was colorless and developed no red color on boiling with conc. HCl. There was therefore no leuco-anthocyanin present.

Purple Cone Type (Tetraploid) from Garden Culture.—(a) *Ethyl Acetate Fraction*.—This was identical with the corresponding fraction from the Red Yellow and Black Yellow types, i.e., an anthoxanthin giving a golden yellow color with dilute KOH but possibly in rather smaller quantities.

(b) *Aqueous Fraction*.—This had a magenta color due to the presence of an anthocyanin. Anthocyanins occur in the plant in combination with various sugar radicles, i.e., as glycosides. A quick method is available² for determining the nature of the glycosides, depending on their partition between two solvents, water and iso-amyl alcohol. On shaking an aqueous solution of the anthocyanin under test with iso-amyl alcohol it may be classified in one of three groups:

(1) *Monoglycosides*.—The anthocyanin is distributed between the aqueous and alcoholic layers, and on dilution with water the concentration of the pigment in the alcoholic layer is *increased*. On saturating with NaCl the pigment is almost entirely taken up by the alcoholic layer.

(2) *Pentose Glycosides*.—On diluting with water the concentration of the pigment in the alcoholic layer is *decreased*, but on saturating with NaCl nearly all the pigment passes into the alcoholic layer.

(3) *Diglycosides and Biosides*.—Very little pigment is taken up by the alcoholic layer and its distribution is but slightly altered on dilution with water or on saturation with NaCl. The anthocyanin of *Rudbeckia* belongs in this class.

Anthocyanins give specific color reactions with various reagents, and can be differentiated on this basis according to Robinson's method (summarized Scott-Moncrieff²). The anthocyanin from *Rudbeckia* gave the color reactions which are summarized as follows:

Sodium acetate	Sodium carbonate	Sodium hydroxide	Ferric chloride
Violet	Violet-blue	Pure blue	Purplish-blue

This combination of color reactions is characteristic of 3-glycosides of cyanidin (i.e., cyanidin in which the hydroxyl at position 3 in the pyran ring is replaced by a sugar molecule). The combined evidence from color reactions and partition between aqueous and iso-amyl alcohol solvents suggests that the pigment is probably cyanidin 3-bioside. To confirm this it will be necessary to examine the hydrolyzed pigment spectrophotometrically and compare its absorption with the known spectral absorption

of cyanidin, and to determine the nature of the sugar residue by standard chemical methods.

Miscellaneous Observations.—There is a suggestion that the Purple and Black Yellow types which contain an anthocyanin and a leuco-anthocyanin, respectively, contain less anthoxanthin than the Red Yellow types. This would be in accordance with general experience in other genera where a negative correlation between anthocyanin and anthoxanthin concentration has been established, leading to the hypothesis³⁻⁴ that both classes of pigments are derived from a common precursor, which is produced in rather limited quantity. In the present case the evidence is not critical since the flower types examined differ in chromosome number, and probably are considerably differentiated genetically as well.

The nature of the anthoxanthin pigment cannot be determined without chemical analysis of larger amounts of material than were used in the present studies since small scale qualitative tests, such as have been developed for anthocyanin determination, are not available. However, since the anthoxanthin extract did not yield an anthocyanin pigment on reduction with zinc and hydrochloric acid, it probably does not contain glycosides of quercetin, which can be reduced to cyanidin under these conditions.

A Chemical Explanation of the "Black Yellow" and "Red Yellow" Reactions with Strong Alkali.—Red Yellow cones were macerated in 8N KOH. The calyces developed a deep orange-red color, and the extract was orange. On acidifying with conc. HCl, the color was discharged. On adding Basic Lead Acetate and boiling, a deep orange precipitate was obtained—a characteristic reaction of anthoxanthins. It is clear that the orange-red color obtained on treating Red Yellow cones with strong alkali is due to the anthoxanthin previously found to be present in the Ethyl Acetate fraction described earlier in this paper. The orange-red color is merely due to the extreme concentration of the anthoxanthin in the calyces of the disc florets; the extract is orange or yellow in color depending on the dilution.

Black Yellow cones yielded a greenish yellow extract on grinding with 8N KOH. This was probably due to a mixture of anthoxanthin (orange-red) and leuco-anthocyanin which had been partially converted to anthocyanin (blue in alkaline solution). Continued grinding failed to leach out any darker pigment, and the color of the calyces faded showing that the pigment was being removed, but the extract remained greenish yellow. Previous experience⁵ with certain flower types in *Gossypium* which contain a leuco-anthocyanin but no anthocyanin, had shown that under certain conditions of extraction the leuco-anthocyanin was unstable, and that when extracts were made by boiling the petals with 1 per cent HCl, the leuco-anthocyanin was partially converted to an anthocyanin, since the extracts gave green colors with alkalis changing to pink on re-acidifying. On the

basis of this previous experience the Black Yellow cone was extracted by boiling with 1 per cent HCl. The extract was cooled and a few drops of strong KOH added. A green color was immediately produced, i.e., the extract behaved as if it contained a mixture of anthoxanthin (yellow) and anthocyanin (blue) pigments. On standing, the blue color faded leaving the golden yellow color characteristic of the anthoxanthin. When the alkaline solution with a green color was acidified by adding conc. HCl drop by drop, the green color turned to pink which also was unstable on standing. A pink color would be expected for an acid solution containing a mixture of anthoxanthin (colorless) and anthocyanin (red). Why the anthocyanin produced from the leuco-anthocyanin should be stable in the plant tissues but unstable on extraction is not known. It seems clear, however, that the black color produced by treating the cones with strong alkali is due to the superposition of orange-red (from the anthoxanthin) on blue (from the converted leuco-anthocyanin), and is therefore in conformity with the pigments obtained in the 1 per cent HCl extracts described earlier.

Conclusions.—The Black Yellow type of *Rudbeckia* is able to produce a leuco-anthocyanin which is concentrated in the central cone of the flower. Cones of the Red Yellow types do not contain a leuco-anthocyanin, but presumably this type carries a gene which is able to convert the leuco-anthocyanin, as the genetic combination, Black Yellow/Red Yellow, is phenotypically purple-coned and contains an anthocyanin. The anthocyanin concerned is probably—subject to confirmation by more detailed methods—a bioside of cyanidin.

Our tests show that there is more than a single gene controlling cone color in *Rudbeckia*. They also give some indications of the chemical steps involved in the formation of the purple pigment. The dominant allele (*RY*) of the red-yellow gene controls an essential chemical step in the gene reactions leading to the production of the leuco-anthocyanin. The dominant allele (*BY*) of the black-yellow gene controls a later step which converts the leuco-anthocyanin to an anthocyanin and its action is therefore dependent on the presence of (*RY*). When the latter is absent, the production of both leuco-anthocyanin and anthocyanin in the flower-cone is blocked. On this basis, it would be expected that the double recessive in a 9:3:4 ratio of an F_2 between black-yellow (*by*) and red-yellow (*ry*) would react as red-yellows. This appears to be the case. Our earlier records¹ show 158 purple-coned plants to 97 with yellow cones in an F_2 population, which is close to the expected 9: 7 ratio. By the use of KOH the yellow-coned types were resolved into 43 *by* to 52 *ry*. The expectation on a 3:4 ratio is 40.7:54.3: ± 3.25 . The agreement is close since the deviation is less than the probable error.

The accompanying diagrams may help in an understanding of the relationships between the different cone colors.

Red Yellow (double recessive)	Red Yellow	Black Yellow	Purple Normal
<i>ryry byby</i>	<i>ryry ByBy</i>	<i>RyRy byby</i>	<i>RyRy ByBy</i>
cannot produce leuco-anthocyanin	cannot produce leuco-anthocyanin	has leuco-anthocyanin cannot produce antho- cyanin	Produces both leuco- and an- thocyanin
	<i>RY</i>		<i>BY</i>
Anthoxanthin	Leuco		Anthocyanin
<i>ry</i> blocks this reaction producing Red Yellows		<i>by</i> blocks this reaction producing Black Yellows	

It seems likely that the chemical mechanism described in this paper, whereby two acyanic mutant flower types produce a cyanic "normal" type in combination, may be of rather general occurrence. Superficially similar cases have been reported in *Lathyrus*,⁶ *Antirrhinum*⁷ and *Cheiranthus*,² and it would be of interest to know whether in these genera also, one of each pair of complementary acyanic flower types contains a leuco-anthocyanin. In *Gossypium* a very similar case has recently been examined⁵ in which, as in *Rudbeckia*, one acyanic flower type carries a gene controlling the presence of a leuco-anthocyanin, and its complementary acyanic type a gene which converts the leuco-anthocyanin to an anthocyanin. Furthermore, unpublished studies have shown that the leuco-anthocyanin in *Gossypium* is intimately related not only to the anthocyanin but also to the anthoxanthin pigments, since its spectral absorption is identical with that of the colorless intermediate reduction product obtained on reducing the anthoxanthin, quercetin, to the anthocyan pigment, cyanidin, *in vitro*. Apart from its intrinsic genetic interest, therefore, it is probable that a continued study of these and similar complementary acyanic flower mutants may lead to the discovery of other blocks in the syntheses of anthocyanins and anthoxanthins and so yield much valuable information on the natural interrelation of these pigments in the plant.

Summary.—The following flower cone types are known in *Rudbeckia hirta*: Purple (*BY RY*), Black Yellow (*by RY*), so-called because florets turn black in strong alkali, and Red Yellow (*BY ry* and *by ry*), which turn red in alkali. Chemical studies of the pigments involved have shown that Purple Cone contains an anthocyan pigment, cyanidin; Black Yellow a leuco-anthocyanin convertible to cyanidin *in vitro*; and Red Yellow contains neither leuco-anthocyanin nor cyanidin. The flower cones of all types contain a yellow anthoxanthin pigment. Genetic and chemical findings are consistent with the hypothesis that the gene, *RY*, is responsible for the production of the leuco-anthocyanin, and the gene, *BY*, for its further conversion to cyanidin. The first step is blocked when *RY* is replaced by *ry*, and the second step is blocked when *BY* is replaced by *by*.

- * Contribution from the Department of Botany, Smith College, New Series, No. 26.
¹ Blakeslee, A. F., *Zeitschr. ind. Vererb.*, **25**, 211-221 (1921).
² Scott-Moncrieff, R., *J. Genet.*, **32**, 117-170 (1936).
³ Robinson, R., *Nature* (London), **137**, 172-173 (1936).
⁴ Lawrence, W. J. C., and Price, J. R., *Biol. Rev.*, **15**, 35-58 (1940).
⁵ Stephens, S. G., *Genetics* (in press).
⁶ Bateson, W., Saunders, E. R., and Punnett, R. C., *Proc. Roy. Soc. London*, **77B**, 236-238 (1905).
⁷ Wheldale, M., *The Anthocyanin Pigments of Plants*, Cambridge University Press, 1916.

ON THE EXISTENCE OF SOLUTIONS OF CERTAIN EQUATIONS IN A FINITE FIELD

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In another paper¹ one of the authors stated that he had arrived at limits, both inferior and superior, for the number of solutions of the equation

$$c_1x_1^{a_1} + c_2x_2^{a_2} + \dots + c_sx_s^{a_s} + c_{s+1} = 0 \quad (1)$$

in the x 's where a 's are integers such that $0 < a < p^n - 1$; $s \geq 2$ for $c_{s+1} \neq 0$ and $s > 2$ for $c_{s+1} = 0$, the c 's being given elements of a finite field of order p^n , p prime, which will be designated by $F(p^n)$; and

$$c_1 \dots c_s x_1 \dots x_s \neq 0. \quad (2)$$

As a consequence of this result, one can obtain

THEOREM I. *The equation (1) with the restriction (2) always has at least k solutions in the x 's for k any given positive integer provided p^n exceeds a certain limit.*

In this paper we shall give two quite different approaches to establish this theorem. The first is closely related to the one previously mentioned and the other argument, although subject to the limitation $s > 2$ when $c_{s+1} \neq 0$, is far simpler and is based on a method^{1a} which was introduced by one of the authors in the study of generalized Gaussian sums over a finite field.

The limit given here can be sharpened, and the proof of this will be published later.

Elsewhere² it was shown that the exact number of solutions of (1) may be determined directly if we know the exact number of solutions of θ^{ma} , θ^{mb} , of the equation

$$\theta^{i+ma} + \theta^{j+mb} + 1 = 0 \quad (3)$$

for each i and j ; θ being a primitive root of $F(p^n)$; $(p^n - 1, a_i) = d_i$, $i = 1, 2, \dots, s$; m is the L.C.M. of d_1, d_2, \dots, d_s . Further in this argument some or all of the a 's may equal $p^n - 1$. To prove this we employed formulae (5), (12) and (15) of the paper just mentioned and all of these contain positive terms only.

We may adapt this method, however, to finding limits from the number of solutions of (1) by first finding limits for the number of solutions of (3) and then employing (5), (12) and (15) of the previous paper.²

In the latter the formulae

$$\begin{aligned} A_{oo} &= (c-1)^2 + c(m-1); & A_{ho} &= A_{ok} = c^2 - c, \\ A_{hk} &= c^2, h \not\equiv k; & A_{hh} &= c^2 - c \\ h &\not\equiv 0 \pmod{m}, k \not\equiv 0 \pmod{m}, & p^n &= 1 + mc \end{aligned} \quad (4)$$

are derived; where

$$A_{hk} = \sum_{i,j}^{0 \text{ to } m-1} [i, j][i+h, j+k]$$

and $[i, j]$ is the number of different pairs r and t in the set $0, 1, \dots, c-1$ which satisfy the equation

$$1 + \theta^{i+rm} = \theta^{j+tm}; \quad p^n - 1 = cm. \quad (5)$$

If $\{i, j\}$ denotes the number of solutions of (3) then we note that

$$[i, j] = \{i, j + \epsilon\}, \quad (6)$$

where $\epsilon = \text{ind}(-1)$, with $x = \theta^{\text{ind } x}$. We have

$$\sum_{i,j}^{0 \text{ to } m-1} (m^2[i, j] - p^n)^2 = \sum_{i,j}^{0 \text{ to } m-1} (m^4[i, j]^2 - 2m^2p^n[i, j]) + p^{2n}m^2$$

which gives, after employing (4) on the right as well as the known relations

$$\begin{aligned} [i, j] &= [j + \epsilon, i + \epsilon] = [-j + \epsilon, i - j] = [i - j + \epsilon, -j] = [-i, \\ &\quad j - i] = [j - i + \epsilon, -i + \epsilon, -i], \end{aligned} \quad (7)$$

$$\left(\frac{p^n}{m^2} + \frac{D}{\sqrt{d}}\right) \geq [i, j] \geq \left(\frac{p^n}{m^2} - \frac{D}{\sqrt{d}}\right), \quad (8)$$

where

$$D = \frac{1}{m} \sqrt{p^n(m^2 - 3m + 2) + 3m + 1}.$$

Here d is the number of different pairs in (7) in the sense that $[h, k]$ is different from $[h_1, k_1]$ if $h \not\equiv h_1$, or $k \not\equiv k_1 \pmod{m}$.

The use of (8) with (6) of this paper and (5), (12) and (15) of the former paper yield superior and inferior limits for the number of solutions of (1), and we obtain a proof of Theorem I.

For the second proof of Theorem I (except for $s = 2$ with $c_{s+1} \neq 0$) we proceed as follows:

Any element α of $F(p^n)$ may be written uniquely in the form, if θ is a primitive root in $F(p^n)$,

$$\alpha = d_0 + d_1\theta + \dots + d_{n-1}\theta^{n-1} = h(\theta) \quad (9)$$

where the d 's are in the included field $F(p)$. Now θ satisfies an irreducible equation $f(x) = 0$ of degree n with coefficients in $F(p)$ and whose roots are $\theta_1, \theta_2, \dots, \theta_n$, with $\theta_1 = \theta$. Set (here tr is short for trace)

$$\sum_{i=1}^n h(\theta_i) = tr h(\theta).$$

We shall now prove

LEMMA 1. If $\zeta = e^{2\pi i/p}$, then, for $n < p$,

$$\sum_{\alpha \in F(p^n)} \zeta^{tr(\alpha t)} = \begin{cases} 0 & \text{for } t \neq 0 \\ p^n & \text{for } t = 0. \end{cases} \quad (10)$$

This statement is obvious for $t = 0$. For $t \neq 0$, αt ranges over all the elements of $F(p^n)$. Hence it is sufficient to prove that

$$\sum_{\alpha \in K} \zeta^{tr(\alpha t)} = \sum_{d_0=0}^{p-1} \dots \sum_{d_{n-1}=0}^{p-1} \zeta^T = \prod_{i=0}^{n-1} \left(\sum_{d_i=0}^{p-1} \zeta^{d_i tr(\theta^i)} \right) = 0, \quad (11)$$

where

$$T = tr(d_0 + d_1\theta + \dots + d_{n-1}\theta^{n-1}).$$

There is one of the i 's such that p does not divide $tr(\theta^i)$. For, if the contrary is true then $tr(\theta^i) = 0$ for all $0 \leq i \leq n-1$. Since $\theta_1 = \theta$, satisfies $(x) = 0$ of degree n as do also $\theta_2, \dots, \theta_n$, then Newton's relations between the elementary symmetric functions of these roots and the sum of the i th powers of said roots give easily $\theta^n = c$, with c in $F(p)$. This yields $\theta^{n(p-1)} = 1$, contradicting the definition of θ as a generator of $F(p^n)$ for $n > 1$, since $p^n - 1 = (p-1)(p^{n-1} + \dots + 1) > n(p-1)$. This proves (10).

We now prove

LEMMA 2. Write K for $F(p^n)$. If

$$\psi(t, a, \zeta) = \sum_{\substack{\alpha \in K \\ \alpha \neq 0}} \zeta^{tr(t\alpha^n)}$$

then for $t \neq 0$,

$$|\psi(t, a, \zeta)| \leq (p^n - 1, a)p^{n/2}. \quad (12)$$

Now

$$\frac{1}{p^n} \sum_{t \in K} (\psi(t, a, \zeta) \psi(t, a, \zeta^{-1})) = \sum_{t \in K} \sum_{\substack{x, y \in K \\ x, y \neq 0}} \zeta^{\text{tr}(tx^n - ty^n)}. \quad (13)$$

Using Lemma 1, the right-hand member reduces to $(p^n - 1)(p^n - 1, a)$, since this is the number of different solutions in x and y , $\neq 0$, of

$$x^a = y^a,$$

in $F(p^n)$. Since $|\psi(t, a, \zeta)| = |\psi(t, a, \zeta^{-1})|$, then (12) gives, if we set $\psi(t, a, \zeta) = \psi(t, a)$,

$$\frac{1}{p^n} \sum_{t \in K} |\psi(t, a)|^2 = (p^n - 1)(p^n - 1, a). \quad (14)$$

Since, if $t \neq 0$, the number of solutions of $ty^a = q$ is $\leq (p^n - 1, a)$, then

$$\begin{aligned} (p^n - 1) |\psi(t, a)|^2 &= \sum_{\substack{y \in K \\ y \neq 0}} |\psi(ty^a, a)|^2, \\ &\leq (p^n - 1, a) \sum_{q \in K} |\psi(q, a)|^2, \\ &\leq (p^n - 1, a)^2 p^n (p^n - 1), \end{aligned}$$

and by (14), we then have Lemma 2. Then for the proof of Theorem 1, we have

$$\begin{aligned} N &= \frac{1}{p^n} \sum_{t \in K} \psi(c_1 t, a_1) \dots \psi(c_s t, a_s) \zeta^{\text{tr}(c_{s+1} t)} \\ &= \frac{(p^n - 1)^s}{p^n} + \frac{1}{p^n} \sum_{\substack{t \in K \\ t \neq 0}} \psi(c_1 t, a_1) \dots \psi(c_s t, a_s) \zeta^{\text{tr}(c_{s+1} t)}, \quad (15) \end{aligned}$$

where N is the number of solutions of (1), and now we count all the solutions under the restriction (2). (In connection with (5) we did not do this exactly, that is, all the solutions of (5) would be $m^2[i, j]$.) Then

$$\left| N - \frac{(p^n - 1)^s}{p^n} \right| \leq \frac{1}{p^n} \sum_{\substack{t \in K \\ t \neq 0}} |\psi(c_1 t, a_1)| \dots |\psi(c_s t, a_s)|,$$

and by Lemma 2, we have

$$\left| N - \frac{(p^n - 1)^s}{p^n} \right| \leq \prod_{i=1}^s (p^n - 1, a_i) \cdot p^{ns/2},$$

whence

$$\frac{(p^n - 1)^s}{p^n} - Ap^{ns/2} \leq N \leq \frac{(p^n - 1)^s}{p^n} + Ap^{ns/2}; \quad (16)$$

$$A = \prod_{i=1}^s (p^n - 1, a_i).$$

The quantity $A > 0$ never exceeds the fixed value $\prod_{i=1}^s a_i$. Set $p^n - 1 = h$. Then the left-hand member of (16) becomes, if $r = (s + 2)/2$,

$$\frac{h^r \left(h^{(s-r)} - A \left(1 + \frac{1}{h} \right)^r \right)}{p^n}, \quad (17)$$

which for $s > 2$ and p^n sufficiently large is obviously $> k$ for k any integer. This proves Theorem I, for $s > 2$. From (16) we may also write

$$N = \frac{(p^n - 1)^s}{p^n} + O(p^{ns/2}). \quad (18)$$

We may now apply Theorem I to congruences with respect to an ideal prime modulus in any commutative ring R with a unity element. We may consider the congruence

$$\alpha_1 x_1^{a_1} + \alpha_2 x_2^{a_2} + \dots + \alpha_s x_s^{a_s} + \alpha_{s+1} \equiv 0 \pmod{\mathfrak{p}}, \quad (19)$$

where now the α 's are fixed elements in R , that is, we may fix the a 's and α 's and consider the above congruence for various values of \mathfrak{p} . This is a bit different from the situation in Theorem I where the domain of the coefficients changes with each \mathfrak{p} . But if the number of incongruent residues (norm) in R of the ideal \mathfrak{p} is finite then the residue classes form a field, since a finite integral domain is a field. Hence we may apply Theorem I and obtain

THEOREM II. *The congruence (19) always has at least k solutions in the x 's for k any positive integer provided*

$$\alpha_1 \alpha_2 \dots \alpha_s x_1 x_2 \dots x_s \not\equiv 0 \pmod{\mathfrak{p}} \quad (20)$$

$s \geq 2$ for $c_{s+1} \not\equiv 0 \pmod{\mathfrak{p}}$ and $s > 2$ for $c_{s+1} \equiv 0 \pmod{\mathfrak{p}}$; \mathfrak{p} is a prime ideal of finite norm in a commutative ring R with a unity element, the a 's are fixed positive integers, the α 's are fixed elements in R , and the norm of \mathfrak{p} is sufficiently large.

If R is the ring of integers in an algebraic field then every ideal has a finite norm so that the Theorem II holds for any algebraic ring of this type with \mathfrak{p} any prime ideal in the ring. If R is the ring of rational integers, then $n = 1$ and R is the system of residue classes modulo p , and we have in particular the

COROLLARY. If in (19) the x 's are rational integers and $\mathfrak{p} = (p)$ with p a prime rational integer then this congruence always has k solutions for p sufficiently large with the other conditions in Theorem II holding, k any integer.

This corollary was given by Mordell¹ when $\alpha_{s+1} \not\equiv 0$, and we include all³ solutions of (19). We considered only solutions prime to \mathfrak{p} (primitive solutions) in our work, following the conditions (2) and (20).

¹ These PROCEEDINGS, 33, 236-242 (1947). In this paper reference to previous results was made, but an important paper by Mordell, *Mathematische Zeitschrift*, 37, 207 (1933) which bore more directly on the contents, was, unfortunately, not mentioned. Other relevant references are Pellet, *Bull. Math. Soc. France*, 15, 80-93 (1886); Dickson, *Crelle*, 135, 181-188 (1909); Hurwitz, *Crelle*, 136, 272-292 (1909); Mitchell, *Ann. Math.*, II, 18, 120 (1917); Davenport, *Jour. London Math. Soc.*, 6, 49-54 (1931); Schur, I., *Jahresber. Deutsch. Math. Verein.*, 25, 114 (1916).

^{1a} Cf. a paper by Hua, Loo-Keng, "On a Double Exponential Sum," soon to appear in the *Science Reports of Tsing Hua University*. An abstract is given in the *Science Record of the Acad. Sinica*, 1, Nos. 1-2.

² These PROCEEDINGS, 32, 47-52 (1946).

³ Mordell's method of proof is different from either of those used in the present paper.

In some ways there is quite a distinction between finding the primitive solutions of an equation in a finite field and finding all solutions. The congruence

$$x_1^2 + x_2^2 + \dots + x_s^2 \equiv 0 \pmod{2}$$

has no solutions in integers prime to 2, if s is odd, but evidently has solutions for some of the x 's even. Again the congruence $x^7 + y^7 + 1 \equiv 0 \pmod{491}$ has no solutions in integers x and y prime to 491. But the congruence $x^7 + 1 \equiv 0 \pmod{491}$ obviously has solutions.

GROUPS, CATEGORIES AND DUALITY

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It has long been recognized that the theorems of group theory display a certain duality. The concept of a lattice gives a partial expression for this duality, in that some of the theorems about groups which can be formulated in terms of the lattice of subgroups of a group display the customary lattice duality between meet (intersection) and join (union). The duality is not always present, in the sense that the lattice dual of a true theorem on groups need not be true; for example, a Jordan Holder theorem holds for certain ascending well-ordered infinite composition series, but not for the corresponding descending series.¹ Moreover, there are other striking group theoretic situations where a duality is present, but is not readily expressible in lattice-theoretic terms.

As an example, consider the direct product $D = G \times H$ of two groups

G and H , together with its canonical homomorphisms $\gamma(g, h) = g$, $\eta(g, h) = h$ into the given factors G and H . The system $[\gamma: D \rightarrow G; \eta: D \rightarrow H]$ consisting of the direct product together with these homomorphisms is characterized, up to isomorphism, by the following property: given any other such system $[\gamma': D' \rightarrow G, \eta': D' \rightarrow H]$ for the same groups G and H , there is one and only one homomorphism $\pi: D' \rightarrow D$ such that $\gamma' = \gamma\pi$, $\eta' = \eta\pi$. Dually, the free product P of groups G and H is the "most general" group generated by subgroups isomorphic to G and H , respectively. This means that there are canonical homomorphisms $\alpha: G \rightarrow P$ and $\beta: H \rightarrow P$ of the factors into the corresponding subgroups of P . This system (P, α, β) is characterized by the following property: given any system $[\alpha': G \rightarrow P', \beta': H \rightarrow P']$ there is one and only one homomorphism $\sigma: P \rightarrow P'$ such that $\sigma\alpha = \alpha'$, $\sigma\beta = \beta'$. The theorem that the direct product of any two groups exists is thus dual to the theorem asserting the existence of the free product. The *proofs* of these two theorems are not dual, but the proofs of many other formal properties are dual, as for instance in the case of the associative law $(G \times H) \times K \cong G \times (H \times K)$. For the direct product D , the canonical homomorphisms γ and η are *homomorphisms onto* their respective ranges G and H ; in the case of the free product P the canonical homomorphisms α and β are *isomorphisms into* P . The "dual" of a theorem about groups and homomorphisms is to be obtained by inverting the direction of each homomorphism, inverting the order of all products of homomorphisms and replacing homomorphisms onto by isomorphisms into.

For abelian groups the duality is more marked. A free abelian group F can be characterized in terms of homomorphisms of abelian groups by the following property:² for any homomorphism $\alpha: F \rightarrow A$ and any second homomorphism $\beta: B \rightarrow A$ onto the image group A there exists a homomorphism $\gamma: F \rightarrow B$ with $\beta\gamma = \alpha$. (The corresponding characterization applies also to free non-abelian groups.) An infinitely divisible abelian group D is one in which there exists for each $d \in D$ and each integer m a solution x of the equation $mx = d$. Any homomorphism of an abelian group A into D can be extended to any abelian group B containing A . This property characterizes the infinitely divisible abelian groups; it may be stated in a form dual to the characteristic property of free groups: given $\alpha: A \rightarrow D$ and an isomorphism $\beta: A \rightarrow B$ of A into B , there exists a $\gamma: B \rightarrow D$ with $\gamma\beta = \alpha$. For an abelian group, free products reduce to direct products. If a factor group of an abelian group is a free group, it is a direct factor. Dually, if a subgroup of an abelian group is infinitely divisible, it is a direct factor.

This duality for abelian groups appears in algebraic topology as a duality between homology and cohomology groups. This phenomenon is especially striking in the axiomatic form of homology theory.³

For locally compact topological abelian groups, the duality phenomena can be formulated explicitly by means of character groups;⁴ each theorem then gives a dual theorem about the character groups of those groups involved in the original theorem. It is instructive to compare this formulation with the duality of plane projective geometry.⁵ A pole-polar reciprocation gives a dual to each projective figure, comparable to the character group of a group. Alternatively, projective geometry has an "axiomatic" or "syntactical" duality: any theorem deducible from the incidence axioms remains true on the interchange of the primitive terms "point" and "line" in the statement of the theorem.

Our objective is a similar formulation of a (partial) axiomatic duality for groups. It clearly must concern the system consisting of all groups and all homomorphisms of one group into another. For certain other investigations of this and similar systems, Eilenberg and the author have introduced the notion of a category.⁶ A *category* is a class of "mappings" (say, homomorphisms) in which the product $\alpha\beta$ of certain pairs of mappings α and β is defined. A mapping e is called an *identity* if $\rho\alpha = \alpha$ and $\beta\rho = \beta$ whenever the products in question are defined. These products must satisfy the axioms:

- (C-1). If the products $\gamma\beta$ and $(\gamma\beta)\alpha$ are defined, so is $\beta\alpha$;
- (C-1'). If the products $\beta\alpha$ and $\gamma(\beta\alpha)$ are defined, so is $\gamma\beta$;
- (C-2). If the products $\gamma\beta$ and $\beta\alpha$ are defined, so are the products $(\gamma\beta)\alpha$ and $\gamma(\beta\alpha)$, and these products are equal.
- (C-3). For each γ there is an identity e_D such that γe_D is defined;
- (C-4). For each γ there is an identity e_R such that $e_R\gamma$ is defined.

It follows that the identities e_D and e_R are unique; they may be called, respectively, the *domain* and the *range* of the given mapping γ . A mapping θ with a two-sided inverse is an *equivalence*.

These axioms are clearly self dual, and a dual theory of free and direct products may be constructed in any category in which such products exist. These axioms do not, however, suffice to express the duality between "homomorphism onto" and "isomorphism into." These notions can be formulated in terms of subgroups and factor groups; with any subgroup $S \subset G$ we can associate the identity injection $i: S \rightarrow G$ of S into G , and with any normal subgroup N of G we can associate the projection $\tau: G \rightarrow G/N$ mapping each element g of G into its coset gN in the factor group G/N . We propose to axiomatize the dual notions "injection" and "projection."

A *bicategory* is a category with two distinguished classes of mappings, the "injections" and the "projections," subject to the following self dual axioms:

- (BC-1). Every identity is both an injection and a projection;
- (BC-2). The product of two injections (projections), when defined, is an injection (projection).

(BC-3). Every mapping γ can be represented uniquely as a product $\gamma = \kappa\theta\pi$, where π is a projection, θ an equivalence and κ an injection.

A mapping of the form $\kappa\theta$ is called a mapping within (isomorphism into); one of the form $\theta\pi$ is called a mapping upon (homomorphism onto).

(BC-4). The product of two mappings within (upon), when defined, is a mapping within (upon).

(BC-5). Two injections (projections) with identical domains and identical ranges are identical.

These concepts suffice to give dual definitions of "subgroups" and "factor groups." Thus e_1 is a "subidentity" of e_2 if there exists an injection with domain e_1 and range e_2 ; this inclusion relation gives a partial order of the identities of a bicategory. We may then define a *lattice-ordered bicategory* as any bicategory in which the subidentities and factor identities of any given identity form a lattice under this partial order.

A group can be interpreted as a lattice-ordered bicategory with an identity; the mappings of the category are all equivalences, and are the elements of the group. A lattice L can be interpreted as a lattice-ordered bicategory in which all mappings are injections: the mappings of the category are the pairs $[a, b]$ with $a \supset b$, and with product $[a, b][b, c] = [a, c]$. Thus the concept "lattice-ordered bicategory" is a common generalization of the notions "group" and "lattice."

We contend that most of the phenomena of universal algebra and of (axiomatic) group duality⁷ have appropriate and simple formulations in terms of lattice-ordered bicategories. In particular, for groups, one may use the lattice-ordered bicategory of all homomorphisms of one group into another. In this category we might interpret projection mapping to mean any (canonical) homomorphism $\tau: G \rightarrow G/N$ of a group G upon its factor group G/N . For this interpretation the product of two projections is not a projection (axiom BC-2 fails). This axiom might be saved by calling a projection any product of such canonical homomorphisms τ , but in this case the projection factor π of any homomorphism is not unique (axiom BC-3 fails).

This apparent difficulty can be surmounted by an attention to fundamentals. A factor group G/N may be described either as a group in which the *elements* are cosets of N , and the *equality* of elements is the equality of sets, or as a group in which the *elements* are the elements of G , and the "equality" is congruence modulo N . Both approaches are rigorous⁸ and can be applied systematically (and with approximately equal inconvenience!) throughout group theory. The difficulties cited disappear when we adopt the second point of view, and regard a group G as a system of elements G with a reflexive symmetric and transitive "equality" relation such that logically identical elements are equal (but not necessarily conversely) and such that products of equal elements are equal.

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¹ Birkhoff, G., "Lattice Theory," *Am. Math. Soc. Colloq. Pub.*, **25**, 48 (1940).

² For the case of abelian groups with operators from a group Q , this property is used in Eilenberg, S., and MacLane, S., "Homology Theory of Spaces with Operators II," forthcoming in *Trans. Am. Math. Soc.*

³ Eilenberg, S., and Steenrod, N., *Proc. Nat. Acad. Sci.*, **31**, 117-120 (1945). (The writer has also profited by reading further unpublished work of these authors on this subject.)

⁴ Pontrjagin, L., *Topological Groups*, Princeton, 1939. Weil, A., *L'Integration dans les groupes topologiques et ses applications*, Paris, 1938.

⁵ Veblen, O., and Young, J. W., *Projective Geometry*, Boston, 1910.

⁶ Eilenberg, S., and MacLane, S., *Proc. Nat. Acad. Sci.*, **28**, 537-543 (1942); *Trans. Am. Math. Soc.*, **58**, 231-294 (1945).

⁷ The formulation with bicategories does not yet indicate the duality between center and factor commutator groups, and similar dual concepts of verbal and marginal subgroups; Hall, P., *J. f. d. reine und angew. Math.*, **182**, 156-157 (1940).

⁸ A careful treatment, emphasizing the equality approach, appears in the unjustly neglected book by Haupt, O., *Einführung in die Algebra*, Leipzig, 1929.

METHODS OF SYMMETRY AND CRITICAL POINTS OF HARMONIC FUNCTIONS

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The most powerful method known for the study of the location of the critical points of harmonic functions is the expression of the gradient of a given harmonic function as the force in a field due to a suitable distribution of matter.¹ Nevertheless simpler methods involving less machinery, based on topological considerations involving symmetry, yield some surprisingly deep results, as we wish to indicate in the present note. Our principal result is

THEOREM 1. Denote by Π_1 and Π_2 the open upper and lower half-planes respectively. Let $u(x, y)$ be harmonic in a region R cut by the axis of reals, and let the relation

$$u(x, y) > u(x, -y) \text{ for } (x, y) \text{ in } \Pi_1 \quad (1)$$

hold whenever both (x, y) and $(x, -y)$ lie in R . Then $u(x, y)$ has no critical point in R on the axis of reals.

Alternate sufficient conditions that $u(x, y)$ have no critical point in R on the axis of reals are that R be bounded by a Jordan configuration B , that $u(x, y)$ be harmonic and bounded in R , continuous in $R + B$ except perhaps for a finite number of points, $u(x, y)$ not identically equal to $u(x, -y)$ in R , and

1. R symmetric in the axis of reals, with $u(x, y) \geq 0$ on $B \cdot \Pi_1$ and $u(x, y) = 0$ on $B \cdot \Pi_2$.
2. R symmetric in the axis of reals, with $u(x, y) \geq 0$ on $B \cdot \Pi_1$ and $u(x, y) \leq 0$ on $B \cdot \Pi_2$.
3. R symmetric in the axis of reals, with

$$u(x, y) \geq u(x, -y) \quad (2)$$

at every point (x, y) of $B \cdot \Pi_1$.

4. (x, y) lies in R whenever $(x, -y)$ lies in $R \cdot \Pi_2$; $u(x, y) \geq 0$ on $B \cdot \Pi_1$ and $u(x, y) = 0$ on $B \cdot \Pi_2$.

5. R' is a region whose boundary B' is symmetric in the axis of reals, and R is a subregion of R' whose boundary in Π_k is denoted by $B' \cdot \Pi_k + B_k$ ($k = 1, 2$), where B_k is disjoint from B' ; we have (2) at every point of $B' \cdot \Pi_1$; we denote by $u_k(x, y)$ the function harmonic and bounded in $R' \cdot \Pi_k$ defined by the boundary values $u(x, y)$ on the axis of reals and on $B' \cdot \Pi_k$; we suppose $u(x, y) \geq u_1(x, y)$ on B_1 , $u(x, y) \leq u_2(x, y)$ on B_2 .

6. R is cut by the axis of reals and $u(x, y)$ has boundary values unity on $B \cdot \Pi_1$, zero on $B \cdot \Pi_2$.

7. R is cut by the axis of reals; the boundary values of $u(x, y)$ on $B \cdot \Pi_1$ are not less than l u b $[u(x, 0)]$ in R ; the boundary values on $B \cdot \Pi_2$ are not greater than g l b $[u(x, 0)]$ in R .

By a *Jordan configuration* we mean a set composed of a finite number of Jordan arcs. As a matter of convention, segments of the axis of reals belonging to B (or B_k) are considered to belong to both $B \cdot \Pi_1$ and $B \cdot \Pi_2$ (or $B_k \cdot \Pi_k$), and boundary values may of course be different on $B \cdot \Pi_1$ and $B \cdot \Pi_2$.

To prove the main part of Theorem 1 we set $U(x, y) = u(x, y) - u(x, -y)$, whence in Π_1 we have $U(x, y) > 0$ and in Π_2 we have $U(x, y) < 0$; on the axis of reals $U(x, 0) = 0$, $\partial U(x, 0)/\partial x = 0$. In the neighborhood of a critical point (x_0, y_0) of $U(x, y)$ of order k the locus $U(x, y) = U(x_0, y_0)$ consists of $k + 1$ analytic Jordan arcs intersecting at (x_0, y_0) at successive angles of $\pi/(k + 1)$; no such arc of the locus $U(x, y) = 0$ can lie in $R \cdot \Pi_1$ or $R \cdot \Pi_2$, so no critical point of $U(x, y)$ lies on the axis of reals in R . On the axis we have $\partial U/\partial x = 0$, whence $\partial U/\partial y \neq 0$, and $\partial U/\partial y > 0$ follows from the behavior of $U(x, y)$ in Π_1 and Π_2 . We also have $\partial U(x, 0)/\partial y = 2 \partial u(x, 0)/\partial y$, so

$$\partial u(x, 0)/\partial y > 0 \quad (3)$$

follows in R .

Both parts 1 and 2 are contained in part 3, so we proceed to establish part 3. Again we set $U(x, y) = u(x, y) - u(x, -y)$, so at every point of $B \cdot \Pi_1$ we have $U(x, y) \geq 0$; at every point of $B \cdot \Pi_2$ we have $U(x, y) = -U(x, -y) \leq 0$. The function $U(x, y)$ does not vanish identically in R

but vanishes on the axis of reals, so we have $U(x, y) > 0$ in $R \cdot \Pi_1$, $U(x, y) < 0$ in $R \cdot \Pi_2$. From the main part of Theorem 1 we now have at any point $(x, 0)$ of R : $\partial U(x, 0)/\partial y = 2\partial u(x, 0)/\partial y > 0$, so (3) follows in R .

In part 4, let B_1 denote the reflection in the axis of reals of $B \cdot \Pi_2$. On both B_1 and $B \cdot \Pi_1$ we have $u(x, y) \geq 0$, and on $B \cdot \Pi_2$ we have $u(x, y) = 0$. Each point of R on the axis of reals lies in a subregion of R symmetric in the axis bounded wholly by points of B_1 and $B \cdot \Pi_2$, so the conclusion follows from part 1. In part 4 examples show that the condition $u(x, y) = 0$ on $B \cdot \Pi_2$ cannot be replaced by the weaker condition $u(x, y) \leq 0$.

To prove part 5 we note that on the boundary of $R' \cdot \Pi_1$ we have $u(x, y) \geq u(x, -y)$, both on $B' \cdot \Pi_1$ and on the axis of reals, whence $u_1(x, y) \geq u_2(x, -y)$ in $R' \cdot \Pi_1$. From the relations $u(x, y) \geq u_1(x, y)$ on B_1 and $u(x, y) \leq u_2(x, y)$ on B_2 we deduce $u(x, y) \geq u_1(x, y)$ in $R \cdot \Pi_1$ and $u(x, y) \leq u_2(x, y)$ in $R \cdot \Pi_2$. In sum we have for (x, y) in $R \cdot \Pi_1$: $u(x, y) \geq u_1(x, y) \geq u_2(x, -y) \geq u(x, -y)$, provided $(x, -y)$ lies in $R \cdot \Pi_2$. Throughout a suitable neighborhood of any point of R on the axis of reals we have (2) satisfied in Π_1 , so the conclusion follows from part 3.

Part 6 is contained in part 7, so we proceed to prove the latter. We set $b_1 = l u b[u(x, 0) \text{ in } R]$, $b_2 = g l b[u(x, 0) \text{ in } R]$, so we have $u(x, y) \geq b_1$ on $B \cdot \Pi_1$, $u(x, y) \geq b_2$ in $R \cdot \Pi_1$, $u(x, y) \leq b_2$ on $B \cdot \Pi_2$, $u(x, y) \leq b_1$ in $R \cdot \Pi_2$. We set $U(x, y) = u(x, y) - u(x, -y)$. If $U(x, y)$ is defined, at a point of $B \cdot \Pi_1$ we have $U(x, y) \geq 0$ and at a point of the reflection of $B \cdot \Pi_1$ we have $U(x, y) \leq 0$; at a point of $B \cdot \Pi_2$ we have $U(x, y) \leq 0$, and at a point of the reflection of $B \cdot \Pi_2$ we have $U(x, y) \geq 0$. Every point $(x, 0)$ in R lies in some subregion R' of R symmetric in the axis of reals bounded wholly by points of $B \cdot \Pi_1$, $B \cdot \Pi_2$, and their reflections; on the boundary of R' in Π_1 we have $U(x, y) \geq 0$ and on the boundary of R' in Π_2 we have $U(x, y) \leq 0$. It is to be noted that the relation $U(x, y) \equiv 0$ in one region R' would imply that relation in every region R' , and throughout R . The conclusion now follows as in the proof of part 3.

In each case under Theorem 1 we have established (3) throughout R ; consequently if each function $U_k(x, y)$ satisfies the conditions of either the main part of Theorem 1 or one of the supplementary parts, with reference to a region R_k , it follows that the function $\sum_1^n \lambda_k U_k(x, y)$, $\lambda_k > 0$, has no critical point on the axis of reals in $R_1 \cdot R_2 \dots R_n$. Indeed we need not require $U_k(x, y) \equiv U_k(x, -y)$ if we have $U(x, y) \equiv U(x, -y)$.

As an illustration of the power of Theorem 1, we prove in detail

THEOREM 2. *Let R be the interior of the Jordan curve C , and let R be provided with a NE (non-euclidean) geometry by means of a conformal map onto the interior of a circle. Let the function $u(x, y)$ be harmonic but not identically zero in R , continuous on $R + C$, non-negative on an arc C_0 of C and zero on $C - C_0$. Then all critical points of $u(x, y)$ in R lie in the sub-region of R bounded by C_0 and the NE line joining the end-points of C_0 .*

Let (x_0, y_0) be an arbitrary point of R not in the subregion of R mentioned. Map R onto the interior of the unit circle $|w| = 1$ so that (x_0, y_0) is transformed into the origin, and rotate the plane so that the image of C_0 lies in the closed upper half-plane. The conclusion now follows from part 1.

Theorem 2 is not new,² and is presented here to show both the range of Theorem 1 and the kind of result that can be proved by its use. For an arbitrary Jordan region R , an arbitrary NE line behaves conformally like an axis of symmetry, for it is the image of such an axis under a conformal map of the interior of a circle onto R . Thus each part of Theorem 1 is of significance in connection with such a map, and a number of the results obtained by mapping are new.

To show the connection of Theorem 1 with fields of force we give an independent proof of

THEOREM 3. *Let the region R be the interior of a Jordan curve C , and let B be a Jordan configuration in R which together with C bounds a region R' . Let the function $u(x, y)$ be harmonic in R' , superharmonic in R , continuous on $R' + C$ and zero on C . Then all critical points of $u(x, y)$ in R' lie in the smallest NE convex region of R containing B .*

Choose C as the unit circle, and choose an arbitrary NE line wholly in R' as the axis of reals, with an arbitrary preassigned point of the NE line at the origin and with B in the upper half-plane. It follows from a classical result due to F. Riesz (1930) on superharmonic functions that $u(x, y)$ can be written in R as

$$u(x, y) = \int_R G(z, t, R) d\mu_t + h(x, y), \quad d\mu_t \geq 0, \quad (4)$$

where $G(z, t, R)$ is Green's function for R with pole in t and $h(x, y)$ is harmonic in R ; since $u(x, y)$ is harmonic in R' , the function $d\mu$ vanishes in R' , and the integral can be taken over $R - R'$ and represents a function harmonic in R' . Of course Green's function can be extended harmonically across C by reflection; at corresponding points the values are the negatives of each other. Consequently the integral converges not merely in the neighborhood of C in R but also in the neighborhood of C exterior to R ; the function represented by the integral is continuous and vanishes on C . It follows from (4) that $h(x, y)$ is continuous on C and vanishes there, hence vanishes identically. Study of the field of force¹ defined as the conjugate of the function $f'(z)$, where $f(z)$ is an analytic function whose real part is $u(x, y)$, now completes the proof. Theorem 3 is contained in part 5 of Theorem 1; the latter requires (notation of Theorem 1) $u(x, y) \geq u_1(x, y)$ on B_1 , which is closely related to the requirement of Theorem 3 that $u(x, y)$ be superharmonic. Riesz's results are of still further significance in connection with part 5 of Theorem 1, where we use both positive and negative mass μ .

We postpone for another occasion the discussion and formulation in

detail of further applications of Theorem 1, but mention that symmetry in a point may be used in a manner similar to our present use of symmetry in an axis. Moreover, still other methods are available for the study of critical points. For instance, topological methods, similar to our proof of the main part of Theorem 1 and involving the consideration of level curves, yield the following two results:

THEOREM 4. *Let the function $u(x, y)$ be harmonic but not identically zero in the region R whose boundary is the Jordan curve J , continuous on $R + J$. Let $u(x, y)$ be respectively non-negative and non-positive on two complementary arcs of J . Then no critical point of $u(x, y)$ lies on the locus $u(x, y) = 0$ in R .*

THEOREM 5. *Let the function $u(x, y)$ be harmonic but not identically zero in the annular region R bounded by the disjoint Jordan curves J_1 and J_2 , continuous on $R + J_1 + J_2$. Let $u(x, y)$ be non-negative on J_1 , non-positive on J_2 . Then $u(x, y)$ has no critical point on the locus $u(x, y) = 0$ in R .*

Theorems 4 and 5 extend to the case where there are admitted other boundary components, disjoint from the Jordan curves already mentioned, on which $u(x, y)$ is assumed to take the boundary value zero.

¹ Walsh, J. L., these PROCEEDINGS, **34**, 111-119, 1948.

² Walsh, J. L., *Bull. Am. Math. Soc.*, **54**, 196-205, 1948.

TUBERCULOSIS IN GERMANY

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A rise in tuberculosis is a usual concomitant of war and prolonged disaster. Germany experienced such an increase in both world wars, as did most countries of Europe. A study of the rise in tuberculosis mortality in Germany, where records of unusual accuracy were maintained in spite of administrative upheaval, is of considerable interest for the general understanding of the epidemiology of the disease.

The analysis here made is based on German documents printed from 1920-1940 and on observations made in a series of visits to Germany since April, 1945, and particularly on studies performed by a commission* appointed by the Secretary of the Army in February, 1948, to "investigate the incidence of and recommend control measures for tuberculosis in the German civilian population."

Like all countries with a continuing rise in the standard of living and simultaneous development of a public health program, Germany experi-

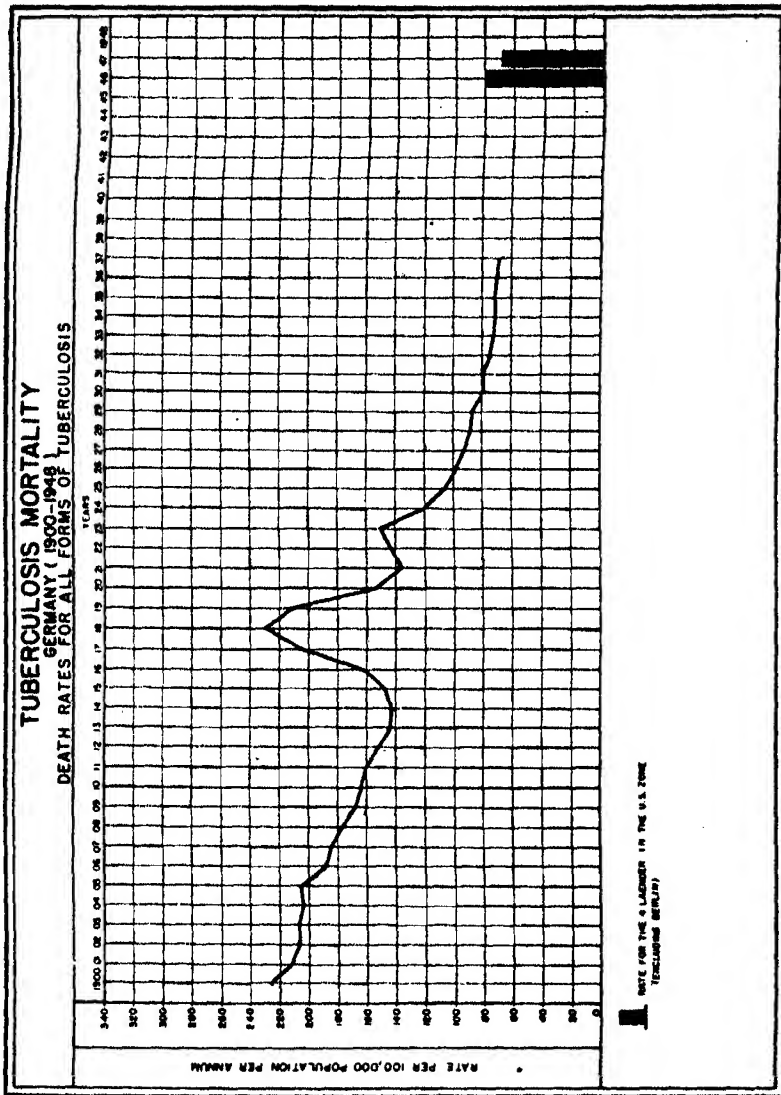


FIGURE 1

enced a steady decline in its mortality from tuberculosis throughout the twentieth century until the advent of World War I. Commencing immediately with the outbreak of hostilities a rise in mortality occurred, which reached its peak in 1918, when a level was attained (in terms of deaths per 100,000 population) approximately the same as that of 1900. With the cessation of hostilities a rapid decline occurred, and by 1921 the rate was again at the prewar level (Fig. 1). In the following year, however, a new

rise occurred which continued for a year and then subsided, after which a continuous decline was maintained until the outbreak of the second world war.

The reasons for the wartime rise and secondary rise in 1922-1923 were much debated by German authorities on tuberculosis. The general conclusion was that each was related to malnutrition. The wartime rise was attributed to the blockade to which Germany was subjected, the cessation of imports from adjacent food-rationed countries, the disruption of the organization for production and crop failures. The second rise was so clearly related to the fantastic inflation of 1922-1923 as not to appear a mere coincidence. That increase was considered due in large measure to the high price of food and resultant impaired nutrition. In each case crowding and excessive exposure to infection were believed by German experts to be of much less moment than nutrition. The significant nutritional defect itself was considered to be an insufficiency of protein and fat.

In the rise in tuberculosis mortality in World War II German authorities again blame malnutrition. Significant differences in the effects of the two wars, however, viz., a much greater destruction of housing and increased hardship in the homeland in the second war, permit a more accurate evaluation of the several factors influencing mortality than was the case after the first world war.

Currency stabilization and resumption of a normal economic state in 1923, with ordinary employment and disappearance of some of the factors supposedly responsible for the 1922 rise in mortality, were accompanied by the development of one of the strongest tuberculosis control programs in the world. In 1938 only a few countries had a lower tuberculosis death rate than Germany. Within Germany excessive rates prevailed in a few places, like Berlin, but the national average was low. A nationwide system of clinics (*Fürsorgestellen*), a generous provision of sanatorium beds, an insurance system financing treatment of all social levels, and a centralized authority for maintenance of a control program (*Reichs Tuberkulose Ausschuss*), all contributed to this success.

With the outbreak of war in 1939 deterioration in the program began. Military mobilization led to a decrease in professional personnel for tuberculosis control, and with the continuation of hostilities, hospital beds once used for tuberculosis were employed for the accumulating casualties. The continuous bombing of German cities was a factor of as yet unmeasured importance. After large raids thousands of injured civilians crowded the hospitals, already undermanned through military necessity.

As time went by, other factors came into play which are believed to have had a seriously adverse effect on the incidence of tuberculosis. Prior to the war Germans with active tuberculosis were not allowed to participate in many forms of domestic and industrial employment. During the war, as

manpower needs grew, documents were published in which the element of contagion was minimized. Germans with active tuberculosis were again permitted employment. It does not appear to be true that they were forced to work, as has been claimed by certain military observers. The former Secretary-General of the Reichs Tuberkulose-Ausschuss is authority for the statement that no coercion was used.†

Manpower needs could not be met from the German civilian population and importation of labor from adjacent allied or occupied countries followed. Little if any screening for tuberculosis was carried out and subsequent critical study has indicated that much tuberculosis was imported in this way. At the end of the war thousands of inmates of the notorious concentration camps, where tuberculosis was rife, were released, and many wandered at will about the countryside, furnishing further opportunity for chance infection.

According to statements made by German authorities serious impairment of nutrition did not occur until the final months of the war. Indeed the statement has been made by Germans that it did not occur until *after* the war, when the reversal of authority, the extra provision for the millions of displaced personnel in the country, and the removal of men suspected of Nazi adherence from productive enterprise, sharply curtailed the German civilian ration.

Availability of housing, on the other hand, was profoundly affected. In some cities, like Berlin, bombing was almost continuous. In others, like Frankfurt and Würzburg, a few saturation raids virtually destroyed the city. In each case only a small fraction of the original houses were left intact. A great concentration of the population occurred in the few remaining homes, with inevitable opportunity for infection of the well by those afflicted with tuberculosis. In the cities continuously bombed, like Berlin and others of great industrial importance, life was under continual strain. Much of it was actually spent in air raid shelters. In one bunker-type shelter in Mannheim it is said that 18,000 people congregated during raids. Such concentrations clearly favored dissemination of the disease.

When Military Government was set up in the spring of 1945 (observations here made apply to the U. S. Zone) the German tuberculosis control program was found badly disorganized. City hospitals and tuberculosis clinics had been destroyed and their functions were being carried on in highly inferior quarters with inadequate equipment. Sanatoria outside of the city were occupied in large measure by wounded soldiers, prisoners of war and non-German displaced personnel. Widespread disruption of transportation was doubly injurious to the program, preventing patients from going to clinics for diagnosis and treatment, making it very difficult for public health nurses and social workers to go to the homes of patients to give advice and home care. As a result, patients who once would have received

prompt hospital and sanatorium care, remained at home. This was soon recognized by German public health authorities as a grave threat to the health of the population.

Data on the prevalence of tuberculosis and mortality rates were untrustworthy in the first few months after the war. One of the principal reasons was an almost complete change in German public health authority. Most of the officials in control had records of Nazi affiliation disqualifying them for further service. An immediate essential step was restoration of a German official tuberculosis control authority in each department of health. Qualified men were hard to find. Data, which once went promptly to a centralized authority in Berlin, after the war could go no further than an inferior health department in a Land. Frequently they did not get that far. Remarkably, essential data were in general preserved at the source, and in time became again available.

A reorganization was affected promptly after military occupation by the Military Government through its Public Health Branch. Continuous effort was made to establish politically acceptable, professionally qualified personnel in responsible positions. As Army combat forces released beds first used for prisoners of war and the housing of displaced personnel, Military Government officers gradually secured more beds for the care of tuberculosis. German barracks which had been made into reasonably effective hospitals during the war become tuberculosis hospitals. Attack on the housing problem by newly constituted German officials, effecting urgently needed repairs in usable buildings, reduced the crowding in homes to some extent and thereby decreased opportunity for contagion. Material assistance was given to the new German economy from available Army stocks. In some German tuberculosis sanatoria a principal source of food was boxed Army K rations. Outpatient clinics were restored to function, although in considerably reduced number. Gradually the sanatoria reverted to their original use, as prisoners of war were released and other provision was made for displaced personnel.

Within a surprisingly short time a remarkably effective tuberculosis control program was again in operation. The total number of beds available for the treatment of tuberculosis, which had reached the low level of less than one per annual tuberculosis death at the end of the war, has approximately doubled since that time and now is not far from the accepted ideal standard ratio of two and a half beds per annual tuberculosis death, with one outstanding exception. In Berlin, a quadripartite-governed city, existing as a municipal island in the Russian Zone, there is a great deficiency in beds for the care of tuberculosis. In former times the sanatoria for Berlin patients were outside the city. This is still true and now they are generally unavailable. An attempt is being made to create an additional

supply of suitable beds in the city, but thus far the program is very far short of the success obtained in the U. S. and British Zones.

Coincidentally with the restoration of a good tuberculosis control program a significant drop in tuberculosis mortality has occurred. Full data on this are included in the official report of the commission to which reference has been made. These will be the subject of later exhaustive analysis and report. For present purposes it will suffice to refer to figure 2 which shows

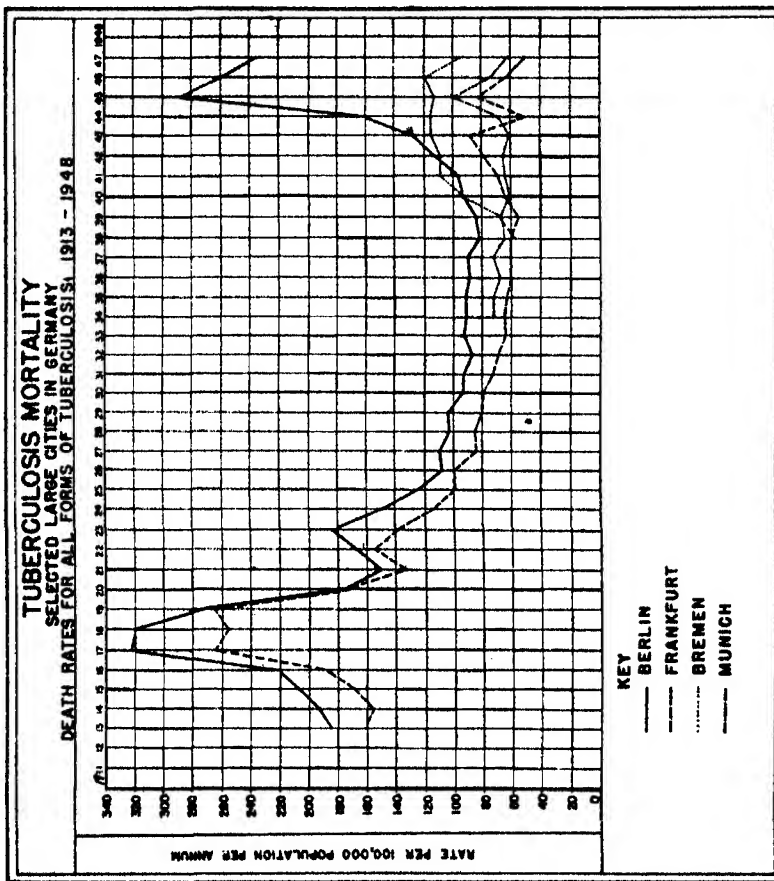


FIGURE 2

the rise and fall of mortality in a representative group of German cities, including Berlin and the three largest cities in the U. S. Zone. It will be noted that the actual levels of mortality reached in the World War I rise were not reached in World War II, but that the percentage increase in mortality over the prewar rate was quite as great. The excessive rate for Berlin, which was at a great disadvantage both before and after the war, is conspicuous.

The drop in mortality since 1945 (1946 in the case of Bremen), when viewed in the light of the drop in 1918, is encouraging, and, it is to be hoped, prophetic. It is admittedly hazardous to conclude that the worst phase is over. The secondary rise in mortality in 1922-1923 shows how susceptible an apparent recovery is to adverse influences. German public health authorities themselves lay less stress on the apparent improvement in death rate than they do on an officially recorded great increase in reported cases of active disease. In the opinion of the commission to which reference has been made this rise in reported cases does not have the full significance attributed to it by some of the German authorities. It appears in large part due to a greatly expanded case-finding program, and is believed to be in some and perhaps large degree affected by the incentive to case reporting furnished by the food supplements granted to persons diagnosed as tuberculous. The available data will be analyzed in detail by the commission. In the meantime the tuberculosis problem, while still of considerable magnitude, appears less serious than was originally feared.

* The members of this commission were Colonel Silas B. Hays, M.C., Major Alonzo W. Clark, M.S.C., Philip E. Sartwell, M.D., and Esmond R. Long, M.D. In Germany the group was joined by Lt. Colonel Charles H. Moseley, M.C. of Office of Military Government, Land Hesse. The statistical data presented in this paper were obtained through the assistance of public health officials in Military Government and German public health officials in Berlin and each Land in the U. S. Zone.

† Personal statement to the author.

SOME RELATIONS BETWEEN THE F 'S AND F^2 'S OF X-RAY DIFFRACTION*

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Introduction.—Last summer, Harker and Kasper¹ showed how Cauchy's inequality could be used to derive some inequalities between the F 's and F^2 's which occur in x-ray diffraction theory. In discussing these important relationships, the writer pointed out that he knew of another avenue of approach to relations between F 's and F^2 's, although no details of the relations had yet been worked out. This alternate approach is through the implication function² which is a transformation of the generalized Harker function.³ The implication has the important property that its maxima correspond to (a) locations of atoms in the corresponding projection of the crystal structure, plus (b) $m - 1$ alternate locations, known as ambiguities (where m is a function of crystal symmetry and

may have values of 4, 3, 2 or 1), plus (c), in some symmetries, "satellites", geometrically related to (a) and (b). This close relation between the maxima of the implication function and the maxima of the electron density function bespeaks an intimate relation between their Fourier coefficients. Since the latter involves F 's and the former F^2 's, it is evident that there is a relation between these which can be found along the lines of implication theory. The present paper outlines the method of finding these relationships, and gives the results in sufficient detail so that they can be used in the determination of phases in crystal structure analysis.

General Relations between F 's and F^2 's.—The general theory underlying the development given here is the following: The phases of diffraction spectra cannot be observed, *but certain differences can be observed*. It can also be shown that the phases are congruent to certain sets of phase differences. To relate F 's to F^2 's, therefore, it is only necessary to determine the moduli of the congruences, and devise methods of isolating the appropriate sets of phase differences. Thus, it is an easy matter to determine x-ray diffraction phases as allowed by congruences. To make the relationships of practical importance to crystal structure analysis, it is also necessary to transform squared amplitudes to amplitudes, and this gives rise to certain complications.

Diffraction from the "Squared Crystal."—Consider a crystal whose cell contains only two atoms which are located at coördinates $x_1y_1z_1$ and $x_2y_2z_2$. The waves diffracted by this crystal are represented by

$$F_{hkl} = f_1 e^{Hx_1 + Ky_1 + Lz_1} + f_2 e^{Hx_2 + Ky_2 + Lz_2} \quad (1)$$

where $H = -2\pi ih$, $K = -2\pi ik$ and $L = -2\pi il$. F_{hkl} represents an amplitude and a phase, and cannot be observed. On the other hand, $|F_{hkl}|^2$ can be observed. This is obtained from F_{hkl} by multiplying it by its complex conjugate, F_{hkl}^* :

$$\begin{aligned} |F_{hkl}|^2 &= F_{hkl} F_{hkl}^* \\ &= (f_1 e^{Hx_1 + Ky_1 + Lz_1} + f_2 e^{Hx_2 + Ky_2 + Lz_2}) (f_1 e^{-Hx_1 - Ky_1 - Lz_1} + f_2 e^{-Hx_2 - Ky_2 - Lz_2}) \end{aligned} \quad (2)$$

$$= f_1^2 + f_2^2 + f_1 f_2 e^{H(x_2 - x_1) + K(y_2 - y_1) + L(z_2 - z_1)} + f_1 f_2 e^{H(x_1 - x_2) + K(y_1 - y_2) + L(z_1 - z_2)} \quad (3)$$

The form of this product is exactly that of the hkl wave scattered by four atoms, two of which are at the origin and have scattering powers f_1^2 and f_2^2 , respectively, the other two having scattering powers $f_1 f_2$ and located at coördinates $(x_2 - x_1)$, $(y_2 - y_1)$, $(z_2 - z_1)$ and $(x_1 - x_2)$, $(y_1 - y_2)$, $(z_1 - z_2)$. An identical wave would have been scattered by a fictitious crystal whose atoms had these scattering powers and coördinates. For convenience, call this fictitious crystal the "squared crystal."

Relations (1)–(3) can be easily generalized by substituting i and j for 1 and 2, and then summing over both i and j . Thus, to each crystal there corresponds a “squared crystal” which has an atom for each pair of atoms in the crystal (including each atom and itself as a pair). The coördinates of the “squared atoms” are the differences of the coördinates of the two atoms in the pair, and their scattering powers are equal to the product of the scattering powers of the atoms of the pair. All of this is a simple and direct consequence of the mathematical rules for forming the complex conjugate. In this way, the F^2 's always involve the differences of phases scattered by pairs of atoms.

The “squared crystal” has atoms at coördinates where the Patterson function^{4, 5} has maxima.

Congruence of Certain Phases with Phase Differences.—For convenience the amplitude factor of the wave is first neglected, the phase relations only being investigated. The relation between the phases of F and F^2 can be found by expanding FF^* for each symmetry, then finding certain collections of terms in F^2 equal of F . This method is blind and extremely patience-trying.

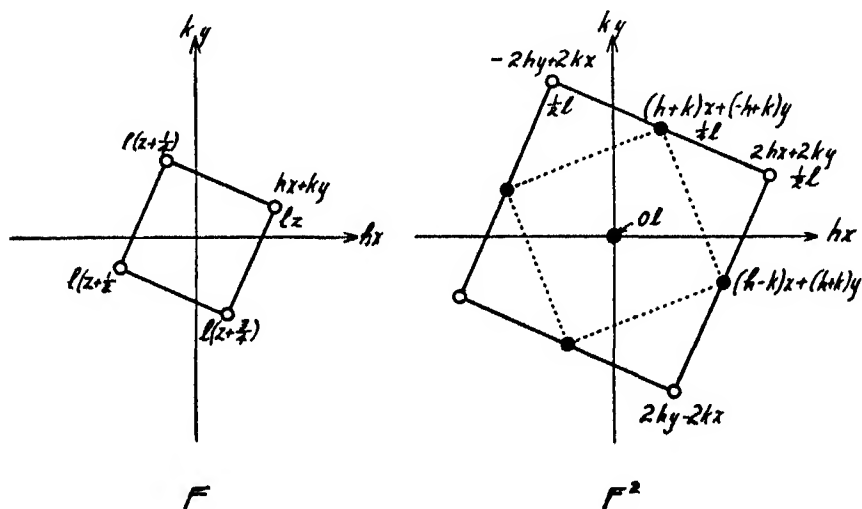


FIGURE 1

A simpler and much more illuminating method is to consider the diffraction phases of F and F^2 (i.e., of crystal and “squared crystal”) as mapped in a space which will be termed *product space*. The coördinates of product space are hx , ky and lz . If an atom is located by these three coördinates in this space, then the sum of its coördinates is the phase of its diffraction hkl , in terms of cycles, since this phase is $e^{-2\pi i(hx+ky+lz)}$. The phase of

F can be visually compared with that of F^2 by comparing the phases scattered by the crystal and "squared crystal." The procedure here is a parallel to that used in comparing the electron density function map with the Harker function map and thus developing the implication theory. In fact, the map of a phase in product space is merely a homogeneous distortion of the map of the atoms producing the phase, as they appear in the crystal. The most useful properties of product space are that (1) linear functions in crystal space remain linear in product space, and (2) the character of hx can be shifted from x to h or the reverse at pleasure.

TABLE 1
ONE SET OF SYMMETRY-EQUIVALENT ELECTRONS

SYMMETRY		RELATIONS IN THE ZONE $\{001\}$			
2	$C_{0, hk}$	$= 2s + s$	$F_{2h, 2k, 0}$		
2 ₁	$C_{1/2, hk}$	$= s$	$F_{2h, 2k, 0}$		
	$C_{0, hk}$	$= 2s$			
3	$C_{0, hk}$	$= 3s + 2s$	$A_{(h-k), (h+2k), 0}$		
3 _{1, 3₂}	$C_{1/2, hk}$	$= 2s^{1/2}$	$A_{(h-k), (h+2k), 0}$		
	$C_{0, hk}$	$= 3s$			
4	$C_{0, hk}$	$= 4s + 2s$	$F_{(h-k), (h+k), 0}$	$+ s$	$F_{2h, 2k, 0}$
4 _{1, 4₂}	$C_{1/4, hk}$	$= 2s^{1/4}$	$F_{(h-k), (h+k), 0}$		
	$C_{1/2, hk}$	$= s$		s	$F_{2h, 2k, 0}$
	$C_{0, hk}$	$= 4s$			
4 ₂	$C_{1/2, hk}$	$= 2s$	$F_{(h-k), (h+k), 0}$		
	$C_{0, hk}$	$= 4s$		$+ s$	$F_{2h, 2k, 0}$
6	$C_{0, hk}$	$= 6s + 2s$	F_{hk0}	$+ 2s$	$F_{(h-k), (h+2k), 0} + s F_{2h, 2k, 0}$
6 _{1, 6₂}	$C_{1/6, hk}$	$= 2s^{1/6}$	F_{hk0}		
	$C_{1/3, hk}$	$= 2s^{1/3}$	$F_{(h-k), (h+2k), 0}$		
	$C_{1/2, hk}$	$= s$		s	$F_{2h, 2k, 0}$
	$C_{0, hk}$	$= 6s$			
6 _{2, 6₄}	$C_{1/2, hk}$	$= 2s^{1/2}$	F_{hk0}	$+ 2s^{1/2}$	$F_{(h-k), (h+2k), 0}$
	$C_{0, hk}$	$= 6s$		s	$F_{2h, 2k, 0}$
6 ₄	$C_{1/2, hk}$	$= 2s$	F_{hk0}	$+ s$	$F_{2h, 2k, 0}$
	$C_{0, hk}$	$= 6s$		$+ 2s$	$F_{(h-k), (h+2k), 0}$
$\bar{3}$	$C_{0, hk}$	$= 6s + 2s$	$F_{(h-k), (h+2k), 0}$		
$\bar{4}$	$C_{0, hk}$	$= 4s + s$	$F_{2h, 2k, 0}$		
$\bar{6}$	$C_{0, hk}$	$= 6s + 2s$	$F_{(h-k), (h+2k), 0}$		

An example of the resemblance of F to F^2 is shown in figure 1 for symmetry 4₁. It is obvious that there is a geometrical similarity between F^2 and F : The level $1/2l$ of F^2 is similar to the projection of F , but has twice the scale, and the level $1/l$ of F^2 is similar to the projection of F , but must be shrunk by a factor $1/\sqrt{2}$ and rotated 45° to bring it into coincidence with F . In this way, F is congruent to certain sections of F^2 with a certain modulus, which is the modulus of the transformation from one figure into the other.

Isolation of Levels of F^2 .—The map of a phase of any symmetrical crystal

contains a figure corresponding to that shown in figure 1 for each atom of the crystal. The map of the phase of the same reflection of F^2 also contains a set of figures, corresponding to those shown in figure 1, for each atom in the original crystal, plus some other points due to interactions between atoms which are not symmetrical. It is necessary to isolate just one layer (with its negative equivalent) in order that the relation of congruence can be drawn between this layer and the projection of F . The isolation can be accomplished by performing a summation $\cos 2\pi lz_1 F^2$ over all values of l , where z_1 is the coördinate of the desired level. It turns out that this gives a coefficient of $s_{z_1} = |\cos 2\pi lz_1|$ to F , and eliminates all other levels, z , of F^2 , since $\sum_{l=-\infty}^{\infty} \cos 2\pi lz$ for other values of z is zero. With the aid of this summation, the relations between the F 's and F^2 's for the various symmetries shown in table 1 may be derived. The values of C_z are shown in table 3.

Complications Due to Scattering Power.—If the crystal contained only a single atom, the transformation of amplitudes of F to F^2 could be made by means of the coefficient R , defined in table 2. When more than one atom is involved, the situation is more complicated, since all atoms do not have equal f 's. If $F_{j, hkl}$ is taken to represent the phase part of the j th equivalent set of atoms, the appropriate correction to take care of this feature is found as follows:

$$\begin{aligned} \sum_j f_{j, hkl}^2 E_{j, hkl} &= \sum_j \frac{f_{j, hkl}^2}{f_{j, h'k'l'}} f_{j, h'k'l'} E_{j, h'k'l'} \\ &= \sum_j R_j f_j E_j \\ &= \sum_j R_j F_j' \\ &= R_1 F_1' + R_2 F_2' + \dots \\ &= R_1 F' + (R_1 F_2' + R_2 F_2' - R_1 F_2') + \dots \\ &= (R_1 F_1' + R_1 F_2' + \dots) + (R_2 F_2' - R_1 F_2') + \dots \\ &= R_1 (F_1' + F_2' + \dots) + (R_2 - R_1) F_2' + \dots \\ &= R_1 F + \sum_j (R_j - R_1) F_j' \\ &= R_1 F + \Delta. \end{aligned}$$

The relations in table 1 can now be transformed into corresponding ones which take account of the scattering coefficients f . The final relations between F and F^2 for the axial symmetries are given in table 2. In this table, P represents the accidental Patterson interaction which occurs on the level z . It can be evaluated if the corresponding implication diagram reveals peaks which are known not to be Harker peaks. This decision can be made in many symmetries because of the absence of characteristic

satellites in the implication. The location of the peak then provides the phase of the $F(P_z)$ and the height of the peak provides the amplitude

TABLE 3
COEFFICIENTS OF THE HARKER FUNCTION

LEVEL IN PATTERSON SPACE	SYMBOL	EXPANSION
0	$C_0 = \sum_l F_{hkl} ^2$	
$1/2$	$C_{1/2} = \sum_{l=2N} F_{hkl} ^2 - \sum_{l=2N+1} F_{hkl} ^2$	
$1/3$	$C_{1/3} = \sum_{l=3N} F_{hkl} ^2 - 1/2 \sum_{l=3N+1} F_{hkl} ^2$	
$1/4$	$C_{1/4} = \sum_{l=4N} F_{hkl} ^2 - \sum_{l=4N+2} F_{hkl} ^2$	
$1/6$	$C_{1/6} = \sum_{l=6N} F_{hkl} ^2 + 1/2 \sum_{l=6N+1} F_{hkl} ^2 - 1/2 \sum_{l=6N+2} F_{hkl} ^2 - \sum_{l=6N+3} F_{hkl} ^2$	

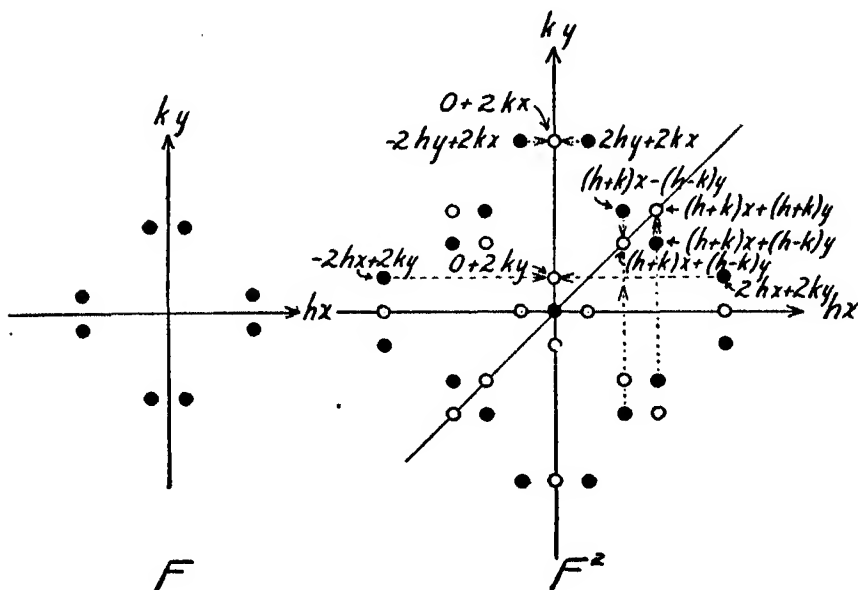


FIGURE 2

Additional Terms Due to Reflection Symmetry.—Every operation of the space group which relates atoms on the level of the implication is represented by a point on the implication. In a similar way, every such operation is represented by a term in the F^2 expansion, such as shown in tables 1 and 2. The forms of these terms for reflection symmetry are readily determined by making use of the properties of product space. In

this space, it is easy to relate the satellites which occur along the line normal to the symmetry plane with the points in general positions. One can then solve for the conditions for which one set of points in the general position become congruent with the satellites. How this is done can be illustrated for symmetry $4mm$ shown in figure 2. In this figure, the points due to reflection (the implication satellites) are shown as open dots, and are found confined to the normals to the reflection planes responsible for them. Study of the figure shows that the outer collection of black dots, which represent $F_{2h, 2k, 0}$ (see Fig. 1), become congruent to the open dots on the vertical line under the condition $h = 0$. Therefore the term due to the reflection plane $(0k0)$ is $F_{2h, 2k, 0}, h = 0$, namely $F_{0, 2k, 0}$. Similarly, the inner collection of black dots, which represent $F_{(h-k), (h+k), 0}$ according to figure 1, becomes congruent to the open dots in the diagonal plane under the condition $k = 0$. Therefore, the term due to the reflection plane $(hh0)$ is $F_{(h-k), (h+k), 0}$ provided $k = 0$, namely F_{hh0} .

TABLE 4
EFFECT OF SATELLITE-PRODUCING SYMMETRY PLANES PARALLEL TO AXES AND THROUGH ORIGIN

AXIAL SYMMETRY NUMBER, n	KIND AND LOCATION OF PLANE	TERMS TO BE ADDED TO AXIAL TERMS
2	Reflection ($h00$)	$S F_{2h, 0, 0} + \Delta_{2h, 0, 0}$
	Glide ($h00$)	$(-1)^h (S F_{2h, 0, 0} + \Delta_{2h, 2k, 0})$
3	Reflection ($h00$)	$S_3 F_{3h, 0, 0} + \Delta_{3h, 0, 0}$
4	Reflection ($h00$)	$S_4 F_{4h, 0, 0} + \Delta_{4h, 0, 0}$
	Reflection ($hh0$)	$S_4 F_{hh0} + \Delta_{hh0}$
6	Reflection ($h00$)	$S_6 F_{6h0} + \Delta_{6h0}$
	Reflection ($hh0$)	$S_6 F_{hh0} + \Delta_{hh0}$

Table 4 contains some of the important reflection relations derived in this way.

Practical Use of the Relations in Crystal Structure Analysis.—The use of these relations in the determination of the phases of certain of the F 's is sketched in another place.⁶ Both the practical importance, and some of the details of the methods of derivation will be treated in detail in subsequent contributions.

¹ Harker, David, and Kasper, J. S., "Phases of Fourier Coefficients Directly from Crystal Diffraction Data," *J. Chem. Phys.*, **15**, 882-884 (1947).

² Buerger, M. J., "The Interpretation of Harker Syntheses," *J. Appl. Phys.*, **17**, 579-595 (1946).

³ Harker, David, "Application of the Three-Dimensional Patterson Method and the Crystal Structures of Proustite, Ag_3AsS_3 , and Pyrargyrite, Ag_3SbS_3 ," *J. Chem. Phys.*, **6**, 381-384 (1938).

⁴ Patterson, A. L., "A Fourier Series Method for the Determination of Interatomic Distances in Crystals," *Phys. Rev.*, **46**, 372-376 (1934).

* Patterson, A. L., "A Direct Method for the Determination of the Components of Interatomic Distances in Crystals," *Z. f. Krist.*, (A) 90, 517-542 (1935).

* Buerger, M. J., "Phase Determination with the Aid of Implication Theory," *Phys. Rev.*, 73, 927-928 (1948).

STABILITY OF THE LAMINAR FLOW THROUGH A STRAIGHT PIPE OF CIRCULAR CROSS-SECTION TO INFINITESIMAL DISTURBANCES WHICH ARE SYMMETRICAL ABOUT THE AXIS OF THE PIPE

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1. *Introduction.*—This investigation grew out of a study made by the writer of the stability of the two-dimensional parabolic laminar flow between parallel fixed walls to infinitesimal disturbances. The result obtained for the parabolic flow which will be reported soon was that, at least as far as α^2 —terms in the wave number α of the disturbance are concerned, the flow is stable to infinitesimal disturbances. Since recent studies of the parabolic flow by Meksyn¹ and Lin,² (where terms in α higher than α^2 have either been neglected *ab initio* or their smallness can be verified *a posteriori*) are at variance with the writer's stability conclusion, inquiries were made as to whether it would be possible to obtain experimental evidence on this point. It turned out that the establishment of a purely two-dimensional parabolic flow is a difficult experimental task, and that the flow through a circular pipe, which happens to be also parabolic, is more amenable to experimental investigation. The writer then undertook an investigation of the stability of the flow through a pipe, and arrived at a stability conclusion for axially symmetrical disturbances. When the investigation was finished it was discovered that Sexl³ had treated the same problem and had arrived at the same conclusion. Upon closer examination it appears, however, that Sexl's argument is incomplete in two respects:

1. Sexl treats only the stability in the two limits of a vanishing Reynolds number R and of large R . The experimental study of the stability of the boundary layer by Schubauer and Skramstad⁴ shows, however, that in the latter case the flow is stable in the limits of $R = 0$ and $R = \infty$, but becomes unstable at intermediate values of R .

2. In his proof of stability for large R , Sexl uses an asymptotic expres-

sion for the solution, which is not sufficiently specified to warrant his conclusion.

In addition, the method used here has the advantage that simple explicit expressions are obtained for the characteristic value C for small R on the one hand, and, asymptotically, for large R , on the other hand, in the relevant region of small α , and that sufficient terms are obtained in each case to allow a satisfactory matching at an intermediate value of R . In this respect, the treatment may claim to a certain measure of simplicity, which is essential⁵ for the ultimate solution of the hydrodynamic stability problem, as otherwise a contribution to this complicated problem may merely serve to elicit a comment such as Rayleigh's:⁶ "The stability problem has further been skillfully treated by von Mises and Hopf. . . . Doubtless the reasoning employed was sufficient for the writers themselves, but the statements of it put forward hardly carry conviction to the mere reader."

2. *The Boundary Value Problems.*—The stability of the laminar flow through a pipe is studied by superimposing on it a disturbance of small amplitude and determining from the hydrodynamic equations and the boundary conditions whether the disturbance grows or decays with time. In a cylindrical system of coördinates r, φ, z the superimposed velocity field is assumed to be of the form $f(r) \exp. [i\alpha(Ct - z)]$, and the flow is considered to be stable to symmetrical disturbances if for all values of the wave number α the imaginary part of $C(=C_i)$ is positive, or unstable if for some values of α , C_i is negative. Actually there exist an infinite set of possible modes for the perturbed flow, and with each mode a characteristic value of C_i , so that a stability proof must embrace all modes, whereas instability is established as soon as it can be shown that for one mode C_i becomes negative.

It will be convenient to introduce the non-dimensional coördinates

$$r' = r/a, \quad z' = z/a, \quad t' = tU/a, \quad R = Ua/\nu, \quad (1)$$

where a denotes the radius of the pipe, U the *maximum* velocity in the laminar flow on the axis of the pipe, and R is the Reynolds number. If we limit the discussion to motion which is independent of the angular coördinate φ , the velocity field can be derived from the potentials,⁷

$$u_r = -(1/r)(\partial\Psi/\partial z); \quad u_z = (1/r)(\partial\Psi/\partial r), \quad u_\varphi = \Omega/r, \quad (2)$$

and the exact equations of motion are

$$\frac{\partial}{\partial t'}(D^2\Psi) + \frac{2\Omega}{r^2} \frac{\partial\Omega}{\partial z} + \frac{1}{r} \left(\frac{\partial\Psi}{\partial r} \frac{\partial D^2\Psi}{\partial z} - \frac{\partial\Psi}{\partial z} \frac{\partial D^2\Psi}{\partial r} \right) + \frac{2}{r^2} \frac{\partial\Psi}{\partial z} D^2\Psi = \frac{1}{R} D^4\Psi, \quad (3)$$

$$\frac{\partial \Omega}{\partial t} + \frac{1}{r} \left(\frac{\partial \Psi}{\partial r} \frac{\partial \Omega}{\partial z} - \frac{\partial \Psi}{\partial z} \frac{\partial \Omega}{\partial r} \right) = \frac{1}{R} D^2 \Omega, \quad (4)$$

where

$$D^2 = \frac{\partial^2}{\partial r^2} - \frac{1}{r} \frac{\partial}{\partial r} + \frac{\partial^2}{\partial z^2}, \quad (5)$$

and the primes in (1) have been dropped. We now write

$$\Psi = \psi_0 + \psi, \quad \psi_0 = \frac{r^2}{2} - \frac{r^4}{4}, \quad U_0 = 1 - r^2, \quad (6)$$

where ψ_0 and U_0 refer to the unperturbed laminar flow, so that the perturbation is derived from ψ and Ω . If we substitute from (6) into (3) and (4), and neglect squares or products in ψ and Ω , we arrive at the equations governing symmetrical *infinitesimal* disturbances of the laminar flow in a circular pipe:

$$\frac{\partial D^2 \psi}{\partial t} + (1 - r^2) \frac{\partial D^2 \psi}{\partial z} = \frac{1}{R} D^4 \psi, \quad (7)$$

$$\frac{\partial \Omega}{\partial t} + (1 - r^2) \frac{\partial \Omega}{\partial z} = \frac{1}{R} D^2 \Omega. \quad (8)$$

Equations (7) and (8) show that in the case of symmetrical infinitesimal disturbances, the perturbation can be of either of two types which are not coupled, namely, motion in concentric circles in planes perpendicular to the axis of the pipe (torsional, Ω) or motion in planes passing through the axis (meridional, ψ). By writing

$$\Omega = G(r) \exp. [i\alpha(Ct - z)], \quad \psi = f(r) \exp. [i\alpha(Ct - z)], \quad (9)$$

$$(d^2 f / dr^2) - (1/r)(df/dr) - \alpha^2 f = g(r), \quad (10)$$

we obtain

$$(d^2 G / dr^2) - (1/r)(dG/dr) - \alpha^2 G + i\alpha R(1 - r^2 - C)G = 0, \quad (11)$$

$$(d^2 g / dr^2) - (1/r)(dg/dr) - \alpha^2 g + i\alpha R(1 - r^2 - C)g = 0. \quad (12)$$

The boundary conditions on G are simply that

$$(G/r) = \text{finite at } r = 0; \quad G(1) = 0. \quad (13)$$

The boundary conditions to be satisfied by f are, from (2),

$$f(0) = \dot{f}(0) = f(1) = \dot{f}(1) = 0, \quad (14)$$

where the dot denotes differentiation with respect to r . Equation (14)

can be converted into an integral condition on g as follows: An appropriate solution of (10) is

$$(1/r)f(r) = AI_1(\alpha r) + \int_0^r [I_1(\alpha r)K_1(\alpha t) - I_1(\alpha t)K_1(\alpha r)]g(t)dt. \quad (15)$$

with

$$(1/r)\dot{f}(r) = A\alpha I_0(\alpha r) + \alpha \int_0^r [I_0(\alpha r)K_1(\alpha t) + I_1(\alpha t)K_0(\alpha r)]g(t)dt. \quad (16)$$

On imposing the boundary conditions (14) at $r = 1$, we find that (14) can be replaced by the condition

$$\int_0^1 I_1(\alpha t)g(t)dt = 0. \quad (17)$$

It is seen from equations (10), (11), (12), (13) and (17) that the characteristic value C depends on the two parameters α and αR . The stability problem resolves itself then into a determination of the sign of C_i in the $(\alpha^2, \alpha R)$ -plane.

3. *Stability of the Laminar Flow through a Circular Pipe to Torsional Perturbations.*—It can be shown directly from the differential equation (11) that the laminar flow is stable to this type of perturbation for all values of the parameters α^2 and αR . Let $x = r^2$, then (11) can be written as

$$\frac{d^2 G}{dx^2} + \left[-\frac{i\alpha R}{4} + \frac{i\alpha R(1-C) - \alpha^2}{4x} \right] G = 0. \quad (18)$$

Multiply (18) by \bar{G} , the complex conjugate of G , and integrate from 0 to 1. We have

$$\int_0^1 G(d^2 G/dx^2)dx = -\int_0^1 \bar{G}\dot{G}dx, \quad (19)$$

because G vanishes at $x = 0$ and $x = 1$. We thus obtain

$$C_r = 1 - [\int_0^1 \bar{G}\dot{G}dx / \int_0^1 \bar{G}\dot{G}(dx/x)], \quad 0 \leq C_r \leq 1, \quad (20)$$

$$C_i = (\alpha^2/\alpha R) + (4/\alpha R)[\int_0^1 \bar{G}\dot{G}dx / \int_0^1 \bar{G}\dot{G}(dx/x)]. \quad (21)$$

Equation (21) shows that C_i is positive for all values of α^2 and αR , and for all modes. This proof, which the writer arrived at independently, was first given by Synge.⁸ Sexl did not recognize the existence of the torsional type of perturbation, but he developed the proof of this section as an auxiliary theorem in his study of the stability of the second type of perturbation. He argues, namely, that since the regular solution of (12) vanishes like r^2 on the axis, the meridional perturbation is stable for such values of α^2 and αR for which it is possible to show that $g(1)$ vanishes to a good approximation. The latter condition obtains whenever the main contribution to the integral in (17) arises from the vicinity of the upper limit.

4. *Stability of the Laminar Flow through a Circular Pipe to Meridional Perturbations.*—(a) *Development for small values of αR .* The meridional perturbation is governed by the system (12) and (17). Let

$$g(r) = rP(r), \quad i\alpha R \equiv \beta. \quad (22)$$

Then (12) and (17) take on the form

$$\frac{d^3 P}{dr^2} + \frac{1}{r} \frac{dP}{dr} + P \left[-\frac{1}{r^2} - \alpha^2 + \beta(1 - r^2 - C) \right] = 0, \quad (23)$$

$$\int_0^1 I_1(\alpha r) r P(r) dr = 0. \quad (24)$$

We shall seek, in the first instance, a development of C which is valid for small β (small Reynolds number):

$$C = (C_{-1}/\beta) + C_0 + \beta C_1 + \dots \beta^n C_n + \dots, \quad (25)$$

and an associated development for P :

$$P = P_0 + \beta P_1 + \dots \beta^n P_n + \dots \quad (26)$$

This can be accomplished by substituting (25) and (26) into (23) and solving successively for the P_n from the differential equations obtained by annulling the coefficient of each power of β . With

$$k^2 = -\alpha^2 - C_{-1}, \quad z = kr, \quad \delta = \left[\frac{d^2}{dz^2} + \frac{1}{z} \frac{d}{dz} + \left(1 - \frac{1}{z^2} \right) \right], \quad (27)$$

one thus finds that

$$\delta P_0 = 0, \quad P_0 = J_1(z), \quad (28)$$

$$\delta P_1 = [(C_0 - 1)/k^2] P_0 + (1/k^4) z^2 P_0, \text{ etc.} \quad (29)$$

These non-homogeneous differential equations can be solved directly with the aid of the identities

$$\delta[z^{n-1} J_n(z)] = 2(n-1)z^{n-2} J_{n-1}(z); \quad \delta[z^{n+1} J_n(z)] = 2(n+1)z^n J_{n-1}(z), \quad (30)$$

$$\delta[z^{n+3} J_n(z) - \{4/(n+2)\} z^{n+2} J_{n+1}(z)] = 2(n+3)z^{n+2} J_{n-1}(z). \quad (31)$$

The result for the first few terms is

$$\begin{aligned} P = J_1 + \beta \left\{ \frac{(C_0 - 1)}{2k^2} z J_2 + \frac{1}{6k^4} z^3 J_2 \right\} + \\ \beta^2 \left\{ \frac{C_1}{2k^2} z J_2 + \frac{(C_0 - 1)^2}{8k^4} z^2 J_2 + \frac{(C_0 - 1)}{12k^6} z^4 J_2 + \right. \\ \left. \frac{1}{360k^8} [36z^5 J_4 - 5z^6 J_4] \right\} + \beta^3 \left\{ \frac{C_2 z}{2k^2} J_2 + \right. \end{aligned}$$

$$\begin{aligned} & \frac{C_1(C_0 - 1)}{4k^4} z^2 J_3 + \frac{C_1}{12k^6} z^4 J_3 + \frac{(C_0 - 1)^3}{48k^6} z^3 J_4 + \\ & \frac{(C_0 - 1)^2}{48k^8} z^5 J_4 + \frac{(C_0 - 1)}{720k^{10}} (46z^6 J_6 - 5z^7 J_8) + \\ & \left. \frac{z^7 J_8}{14k^{12}} - \frac{z^8 J_7}{60k^{12}} + \frac{z^9 J_8}{1296k^{12}} \right\} + \dots, \quad (32) \end{aligned}$$

where we have written J_n for $J_n(z)$.

Substituting now (32) into (24) we obtain from the first term

$$\int_0^1 r J_1(kr) I_1(\alpha r) dr = [\alpha I_2(\alpha) J_1(k) + k J_2(k) I_1(\alpha)] / (\alpha^2 + k^2) = 0, \quad (33)$$

an equation which serves to determine k , and with it C_{-1} , as a function of α . The second term in (32) yields

$$C_0 = 1 - (1/2) [\int_0^1 r^4 I_1(\alpha r) J_2(kr) dr / \int_0^1 r^2 I_1(\alpha r) J_2(kr) dr]. \quad (34)$$

These are rather complicated expressions, but useful information can be obtained from them by expanding k and the C_n into power series in α^2 , as follows:

$$J_2(\kappa) = 0, \quad (35)$$

$$k^2 = \kappa^2 - (1/2)\alpha^2 + \dots, \quad (36)$$

$$\begin{aligned} C = & \left\{ \frac{i\kappa^2}{\alpha R} + \left(\frac{2}{3} + \frac{4}{\kappa^2} \right) + \left(-\frac{1}{45\kappa^2} + \frac{4}{\kappa^4} - \frac{88}{\kappa^6} \right) i\alpha R + \right. \\ & \left. \left(\frac{2}{945\kappa^4} - \frac{20}{7\kappa^6} + \frac{880}{3\kappa^8} - \frac{5792}{\kappa^{10}} \right) \alpha^2 R^2 + \dots \right\} + \alpha^2 \left\{ \frac{i}{2\alpha R} + \right. \\ & \left. \left(\frac{1}{6\kappa^2} - \frac{8}{\kappa^4} \right) + \left(\frac{1}{15\kappa^4} - \frac{62}{3\kappa^6} + \frac{512}{\kappa^8} \right) i\alpha R + \dots \right\} + \\ & \dots 0(\alpha^4). \quad (37) \end{aligned}$$

Although only α^2 -terms have been retained in (36) and (37), their contribution is so small that even at $\alpha = 1$ accurate values for k and C can be obtained. Thus, at $\alpha = 1$ the exact value for k , as determined from (33), is 5.089, whereas (36) yields 5.087; also, the exact value of C_0 determined from (34) is at $\alpha = 1$ equal to 0.8134, compared to a value of 0.8131 obtained from the second terms in each of the braces in (37).

(b) *Development for large values of αR .* With $x = r^2$, equation (12) becomes

$$4x(d^2g/dx^2) + [-\alpha^2 + i\alpha R(1 - x - C)]g = 0, \quad (38)$$

whose solution which is regular at the origin is

$$g = e^{-\tau x} F(a, 2, 2\tau x); \quad \tau = (1/2)\sqrt{\alpha R} e^{i\pi/4}, \quad (39)$$

$$a = 1 - \frac{(1-C)\sqrt{i\alpha R}}{4} + \frac{\alpha^2}{4\sqrt{i\alpha R}}. \quad (40)$$

The integral condition (17) takes on the form

$$\int_0^1 I_1(\alpha r) e^{-\tau r^2} r^2 F(a, 2, 2\tau r^2) dr = 0. \quad (41)$$

For large values of αR the real part of τ becomes large, and is positive, so that the following asymptotic expansion holds

$$e^{-\tau x} F(a, 2, 2\tau x) \rightarrow \frac{x e^{\tau x} (2\tau x)^{a-2}}{\Gamma(a)} \left\{ 1 + \frac{(1-a)(2-a)}{2\tau x} + \dots \right\}. \quad (42)$$

This expression is appropriate if, when substituted in (41), it yields a value for a such that the term $(1-a)(2-a)/(2\tau x)$ actually becomes small for large τ . This is indeed the case, since because of the $e^{\tau x}$ factor and the monotone character of $I_1(\alpha r)$ the principal contribution to the integral in (41) arises from a small region near the upper limit, with the result that we must have

$$1/\Gamma(a) = 0, \quad a = -n, \quad n = 0, 1, 2 \dots \quad (43)$$

as would follow by carrying out a partial integration. It follows from (40) that in the limit of large αR the characteristic value of C_n of the n -th mode has the asymptotic form

$$C_n = 1 - \frac{4(1+n)e^{-i\pi/4}}{\sqrt{\alpha R}} + \frac{i\alpha^2}{\alpha R}. \quad (44)$$

Since the imaginary part of C_n in (44) is positive, this result implies stability at large αR for all modes, the damping increasing with the order of the mode. We note also that for large Reynolds numbers all modes are propagated with the maximum speed on the axis of the pipe.

Sextl did not recognize that the regular solution of (12) is given by (39) which behaves asymptotically according to (42). Instead, he used (*loc. cit.*, page 813) a linear combination of the standard asymptotic solutions of (12):

$$g^*(r) = r^{1/2} (1 - r^2 - C)^{-1/4} [\exp. \int_n^r \sqrt{-i\alpha R(1 - r^2 - C)} dr + K \exp. - \int_n^r \sqrt{-i\alpha R(1 - r^2 - C)} dr], \quad (45)$$

with the constant K left undetermined. He then states that the integral in (41) becomes equal to $g^*(1)I_1(\alpha)$, a result which was obviously arrived

at by partial integration. This step would be justified if it could be shown that $g^*(1)$ becomes large, as we have done in (42) for $g(1)$. But with K left arbitrary the possibility is not excluded that the solution of (12) which is regular at the origin is represented near $r = 1$ by either the positive exponential term in (45) alone, or by the negative exponential alone, in which case $g^*(1)$ may actually vanish. We therefore do not regard this part of Sexl's argument as complete.

(c) *Development for intermediate values of αR .* Though the explicit expressions for the characteristic value C given in (37) and (44) assure stability in the limits of small Reynolds numbers on the one hand and large Reynolds numbers on the other hand, we must still investigate the behavior of C in the intermediate region of αR . We shall do this for the first mode by deriving a correction term to (44), and then showing that at $\alpha R = 200$ the resulting expression yields a value for C which is in good agreement with the value derived from (37), the difference being of the order of the last terms used in each. For the first mode we have

$$a \rightarrow 0, F(a, 2, z) \rightarrow 1 + af(z) + O(a^2), \quad (46)$$

$$f(z) = \int_0^1 (e^z - 1 - z)(dz/z^2) \rightarrow (e^z/z^2)[1 + (2/z) + (3!/z^2) + \dots], \quad (47)$$

$$\int_0^1 e^{-\tau z} x f(2\tau x) dx \rightarrow (e^\tau/4\tau^3)[1 + (2/\tau) + \dots]. \quad (48)$$

Substituting (46) and (47) into (41), and using, in the first instance, only the leading term in

$$I_1(\alpha r) = (\alpha r/2)[1 + (\alpha^2 r^2/8) + (\alpha^4 r^4/192) + \dots], \quad (49)$$

we get

$$\int_0^1 g(x) dx \rightarrow (1/\tau^2) + a(e^\tau/4\tau^3)[1 + (2/\tau) + \dots] = 0, \quad (50)$$

$$a \rightarrow -4\tau c^{-1}[1 - (2/\tau) + \dots], \quad (51)$$

with the understanding that it is to be used only when τ is sufficiently large to make $a \ll 1$. By a similar treatment one can obtain an additional correction term to a arising from the α^2 -term in the bracket of (49). We thus obtain for the characteristic value C_1 of the first mode

$$C_1 = 1 - \frac{2}{\tau} + \frac{i\alpha^2}{\alpha R} - 8e^{-\tau}\left(1 - \frac{2}{\tau}\right) + \alpha^2 e^{-\tau}\left(1 - \frac{1}{\tau}\right) + \dots, \quad (52)$$

where the first three terms are taken from (44). As a check, we evaluate C_1 from (37) and (52) for the case $\alpha^2 = 0$, $\alpha R = 200$. From the former we get a value of $(0.805 + 0.154i)$, while the latter yields $(0.798 + 0.156i)$, the term $8e^{-\tau}$ having the value of $(0.015 + 0.052i)$.

We have thus shown that when the parameter α^2 vanishes, equations

(37) and (52) jointly cover the whole range of αR . The α^2 -term in (37) is small numerically and, furthermore, makes a positive contribution to the damping in the case of the first mode. For large αR the contribution from the α^2 -term in (52) is also small, since it vanishes exponentially like $e^{-\tau}$. It can be shown by using the same procedure as above, that for moderate values of α , the α^2 -term in (52) is replaced by

$$-\frac{4\alpha}{I_1(\alpha)} \exp. \left[-\tau + \frac{4\alpha^2}{\tau} \right] \cdot \left\{ 1 + \frac{1}{\tau} \left[-3 + \frac{\alpha I_0(\alpha)}{2I_1(\alpha)} \right] + \dots \right\}, \quad (53)$$

again provided this term is small. For large values of α

$$I_1(\alpha x) \rightarrow (e^{\alpha x} / \sqrt{2\pi\alpha}) [1 - (3/8\alpha) + \dots], \quad (54)$$

and the last two terms in (52) are replaced by the even smaller term of $-8\tau e^{-2\tau}$. It appears, therefore, that *for large Reynolds numbers the damping of waves of finite wave number approaches the values for vanishing wave number.*

5. *Summary.*—Infinitesimal and axially symmetrical disturbances of the laminar flow through a pipe of circular cross-section fall into two classes which are not coupled: torsional and meridional. In the torsional perturbation the flow is in concentric circles in planes perpendicular to the axis. The laminar flow in a circular pipe is stable to this type of disturbance at all Reynolds numbers, a result which was first shown by Synge.⁶ In the meridional perturbation, the flow is in planes passing through the axis. The stability to meridional perturbations was studied by Sexl,⁷ but his treatment is shown to be incomplete. In this investigation an explicit expression is derived for the characteristic value in the case of a meridional disturbance which is valid for small Reynolds numbers R (equation 37). An asymptotic expression for C , valid for large R , is also derived in equation (44). Additional terms are added to the latter in equation (52) for the first mode, which has the lowest damping. In the case of vanishing wave number α of the perturbation, but finite αR , equations (37) and (52) match at $\alpha R = 200$ and are, therefore, sufficient to cover jointly the whole range of αR from 0 to ∞ . In this limit, the first mode in meridional disturbances is, therefore, demonstrated explicitly to be damped. For large R all modes are propagated with a velocity approaching the maximum velocity on the axis of the pipe.

The α^2 -terms in (37) and (52) make a relatively small contribution to C , even at $\alpha = 1$, and, furthermore, in the case of the first mode they *increase* the damping. It is shown that also when α is moderate or large, C approaches its value for $\alpha = 0$ in the limit of large Reynolds numbers. The difference in C between the case $\alpha = 0$ and a finite α becomes, except for a positive imaginary term ($i\alpha^2/\alpha R$), of the order of $\exp.(-A\sqrt{R})$.

The conclusion is, therefore, drawn that the laminar flow in a pipe of circular cross-section is stable to infinitesimal meridional disturbances.

Although our discussion has been confined to axially symmetrical disturbances only, it is of interest that the experimental evidence on the breakdown of the laminar flow in a pipe of circular cross-section indicates that the so-called critical Reynolds number can be increased, apparently indefinitely, by minimizing the amplitude of the disturbances. The classical stability investigations of Reynolds⁹ were performed on the flow in a circular pipe, and already then his experiments on this and other types of flow led him to distinguish between cases where instability sets in at a critical value of R for *infinitesimal* disturbances and cases, like the flow in a pipe, where apparently only disturbances of *finite amplitude* can cause a breakdown. While it is possible to cite in this connection the following quotations from Reynolds' paper (p. 75): "But the fact that in some conditions it will break down for a large disturbance while it is stable for a small disturbance shows that there is a certain residual stability so long as the disturbances do not exceed a given amount." "But the general impression left in my mind was that . . . as though disturbances in the tank, or arising from irregularities in the tube were necessary to the existence of a state of instability," it is clear that by its very negative nature it is impossible to prove experimentally that stability exists for all Reynolds' numbers. In the summer of 1910 Ekman¹⁰ repeated Reynolds' experiments in the original apparatus, taking extra precautions to minimize the disturbances. He succeeded in increasing Reynolds' critical value of 1.3×10^4 by a factor of 4, and concluded that: "On the other hand, the experiments are, as already mentioned, in favor of the opinion that there is no critical velocity at which approximately rectilinear flow changes into turbulent; but this velocity always increases with decreasing disturbances."

NOTE: Since writing this paper it was pointed out to the author by Professor G. I. Taylor that with a fixed pipe-length of only 140 cm. the flow in Ekman's experiments at the high Reynolds numbers was not of the fully developed parabolic type, but rather of the boundary-layer type characteristic of the inlet. The highest Reynolds number for which the parabolic flow in a circular pipe was proven to be stable is 3.2×10^4 in Taylor's experiments (see reference 7, page 321), where the pipe was long enough to assure a fully developed parabolic velocity distribution.

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THE MECHANISM OF FLOW FOR SOLID METALS*

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Solids show a continuously increasing deformation if subjected to a load of suitable magnitude for a sufficient time. If rupture does not occur, a process of relaxation causes the rate of flow with constant stress to approach a final constant value. Rupture finally occurs in a stressed material whenever bonds are broken by flow faster than they mend. Whenever the stress on a flowing unit is relaxed, the equilibrium distribution of surrounding units is disturbed. Consequently, the exterior stress will cause a succession of adjustments to occur until equilibrium is restored.

In order that an atom, molecule or group of molecules may move from one position of equilibrium to another, it is necessary that space be provided into which the molecules or atoms can jump. If one thinks of the holes and other imperfections in a system, the following idealized classifications of solid, liquid and gas can be made. A perfect solid (Fig. 1(a)) at absolute zero temperature is a system in which all lattice sites are filled and the material has a continuum of perfect order throughout. This ideal situation is never quite realized since actual solids contain holes and a variety of distortions even at absolute zero. At any temperature greater than zero and less than the temperature of melting a section through the solid will show empty sites (holes) as well as regions of higher than average density, as shown in figure 1 (b). In this case the empty sites, or holes, occur infrequently and are not mutually connected. The important concept, that of long range order, is that a continuum of nearly perfect order still exists extending at least as far as the boundaries of the mosaic block.¹ Within this continuum of order there are enclosed domains of imperfections (having liquid properties) which, due to thermal fluctuations, are short-lived and are "recrystallizing" while other such domains alternately reappear at other positions in the domain of rigidity.

Above the temperature of melting (Fig. 1 (c)) the substance is liquid, characterized by a discontinuity of the nearly perfect order and an increase

of holes. The continuum of perfect order has now been so diminished that it exists only as islands in a continuum lacking long-range order.

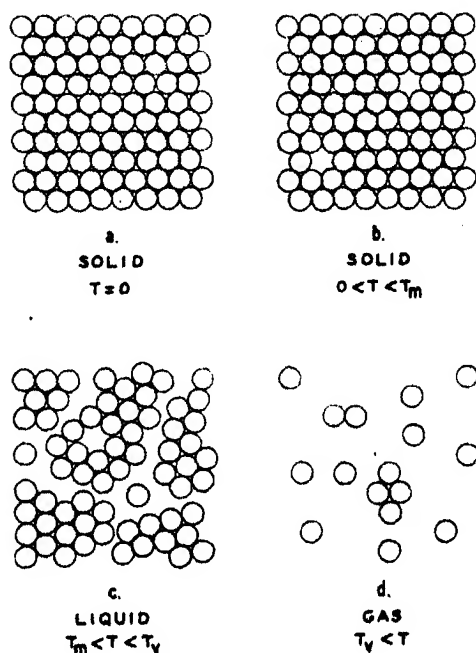


FIGURE 1

Idealized mosaic block structure of solid, liquid and gas.

In the gaseous state there is a prevalence of empty sites and atomic aggregates occur only rarely and are localized. The gas phase is unique in that the empty sites or holes form the continuum surrounding the isolated atoms or molecules and, infrequently, very small regions of perfect order.

Flow of material can occur only by the movement of atoms or molecules into holes, or, conversely, the re-distribution of holes or imperfections through a medium. Imperfections in solid materials may be classified into the following general types.

A. Non-equilibrium imperfections of three classes: (a) Imperfections due to the presence of foreign material such as gas in the form of minute bubbles, refractory particles in glass or carbide inclusions in steel. In a broad sense surface scratches or indentations should be considered as unenclosed holes.

(b) Imperfections due to the inhomogeneity of the material resulting from poor mixing of the constituents or insufficient processing. Such localities produce strain or internal cracks because of the differing coefficients of thermal expansion of the material as a function of composition.

(c) Structural imperfections may be caused by two effects. When cooled too rapidly, a material such as an alloy quite frequently will produce lattices having vacant atomic sites. The second effect, often found in glasses or solids of complex compounds such as the mixed oxides of metals, results in a structural bridgework typified by dumping a load of bricks into a box, thereby producing cavities. Molten oxides, when quenched, collapse from the liquid state and are "frozen" into non-equilibrium structures containing bridged voids. In some substances, particularly metals, rapid cooling also causes a network of voids from the lack of cohesion of the crystallites grown from many nuclei. It is interesting to note that the "frozen" or bridgework, holes common to glass are usually enclosed by the matrix, while the bulk of the metallic voids or intercrystalline boundaries enclose metallic units.

B. Equilibrium imperfections: These imperfections are characterized by the fact that they exist independently of man's attempts to eliminate them without modifying the thermodynamic variables such as temperature and pressure. In general, they differ from the non-equilibrium holes in that they are smaller and represent "short-range" and often "short duration" inhomogeneities. There are two important kinds of equilibrium imperfections: (a) those arising from fluctuations in composition, and (b) those arising from thermal fluctuations in density.

(a) It can be proved statistically that no amount of stirring of the ingredients in an alloy mixture or a glass batch prior to melting could possibly cause perfect homogeneity of the mixture, or batch, particles. When melting occurs, the attainment of composition homogeneity becomes easier because the problem is changed from the mixing of grains to the mixing of atoms or molecules. The fluctuations are of very small size, not only because of the smallness of the atoms or molecules, but because of the fact that the mutual attractions tend to force the atoms into preferred localities. Any inhomogeneity prevailing after solidification may result in the occurrence of holes which are created by regional variations in the coefficient of thermal expansion as a function of composition.

(b) According to the simplest picture, any substance in equilibrium is uniform and not subject to spontaneous changes. However, a statistical viewpoint permits the calculation of local deviations from uniformity. Motion of these local deviations in density, as we shall see in the case of metals, provides the principal mechanism for the release of stress.

Of all the imperfections discussed above, those created by fluctuations in density are the least understood and are to be considered in this paper.

The problem of density fluctuations as evidenced by light scattering has been studied by Lord Rayleigh² and many others,³ and the foundation for the theory has been firmly established.

Fluctuations in density are given by the relation:⁴

$$\left(\frac{\Delta\rho}{\rho}\right)^2 = -\frac{k}{Mv^2(\partial^2s/\partial v^2)_E} \quad (1)$$

where

ρ = density

v = molal volume in a small selected portion of the material

M = mass of the small portion

s = specific value of entropy

k = Boltzman constant = 1.38×10^{-16} erg. deg.⁻¹

E = internal energy

From the thermodynamic expression

$$T dS = dE + P dv$$

one immediately obtains

$$(\partial s/\partial v)_E = P/T$$

For the second derivative a straightforward calculation yields

$$(\partial^2s/\partial v^2)_E = 1/T(\partial P/\partial v)_E - P/T^2(\partial T/\partial v)_E \quad (2)$$

where s , v , E have been defined, and

P = pressure

T = temperature in °K.

Equation (2) may be written in the form

$$(\partial^2s/\partial v^2)_E = -\frac{C_P - Pv\left(2\alpha - \frac{P\beta}{T}\right)}{C_vTv\beta} \quad (3)$$

where

$\beta = -1/v(\partial v/\partial P)_T$ = coefficient of compressibility

$\alpha = 1/v(\partial v/\partial T)_P$ = coefficient of thermal expansion

C_P = heat capacity at constant pressure

C_v = heat capacity at constant volume

For solids $C_P \approx C_v$ and equation (3) reduces to

$$(\partial^2 s / \partial v^2)_T = -\frac{1}{Tv\beta} + \frac{P \left(2\alpha - \frac{P\beta}{T} \right)}{C_v T \beta} \quad (4)$$

To a good approximation, the second part of the right-hand member of equation (4) can be neglected and equation (1) can be written as

$$\overline{\left(\frac{\Delta \rho}{\rho} \right)^2} = \frac{kT\beta}{Mv} = \frac{kT\beta}{V} \quad (5)$$

a well-known result.⁴

The immediate problem is to apply equation (5) to observed data.

Application.—The flow of metals in tension has been carefully analyzed in a previous paper.⁵ An equation to express the rate of flow with relation to structural and thermodynamic parameters was seen to have the following form:

$$\dot{\epsilon} = 1/g \cdot \dot{\sigma} + 2V_h/V_D \cdot kT/h \cdot e^{-\frac{\Delta F^*}{RT}} \cdot \sinh(V_h \sigma / 2kT) \quad (6)$$

where

- $\dot{\epsilon}$ = true strain rate
- σ = stress (applied)
- $\dot{\sigma}$ = stress rate
- V_h = volume of hole
- V_D = volume of flow unit
- ΔF^* = free energy of activation
- g = spring modulus
- k = Boltzman constant
- R = gas constant
- h = Planck's constant (6.62×10^{-27} erg. sec. deg.⁻¹)
- T = temperature (°K.)

From the analysis of the behavior of brass and SAE 1020 steel in tension it was determined that ΔF^* was an almost pure entropy term and that for temperatures not excessively high, $\Delta F^*/T$ was independent of temperature as shown in figure 2. It is now of interest to see how these facts may be understood in terms of fluctuations in density. Rewriting equation (5) as

$$\overline{\left(\frac{\rho}{\Delta \rho} \right)^2} = \frac{V(1/\beta)}{kT} \quad (7)$$

and from the definitions

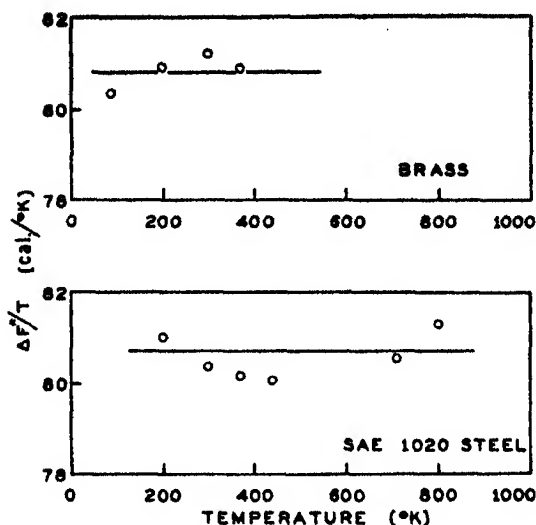


FIGURE 2

Curves showing $\Delta F^*/T$ versus temperature relationship.

$$(\partial F/\partial P)_T = V; \quad \beta = -1/V(\partial V/\partial P)_T$$

one obtains

$$-(\partial F/\partial P)_T(\partial P/\partial V)_T \cdot V = -(\partial F/\partial V)_T \cdot V = \Delta F$$

and from this, equation (7) can be written as

$$\left(\frac{\rho}{\Delta\rho}\right)^2 = \frac{V(1/\beta)}{kT} = \frac{\Delta F^*}{kT} = \text{const.} \quad (8)$$

It is apparent from the constancy of $\Delta F^*/T$ for the metals investigated that neither the nature of the substance nor a difference in mechanism affects the value of $\left(\frac{\rho}{\Delta\rho}\right)^2$ since by (8) we take this equal to $\Delta F^*/kT$. In

other words, these results suggest that since the expression $\left(\frac{\rho}{\Delta\rho}\right)^2$ is a constant we should think of the process of activation as the crowding together of some molecules (and the consequent rarefaction of others) creating the holes or imperfections necessary for the flow process.

Another result obtained from the analyses of metals was the fact that V_h , the volume of the hole into which the flowing unit jumps, was temperature dependent and that V_h/T is approximately a constant as shown in Fig. 3. If we identify V_h with the amount of material whose density is fluctuating at any time,[†] we obtain the relation

$$\frac{V_h(1/\beta)}{kT} = \text{const.} \quad (9)$$

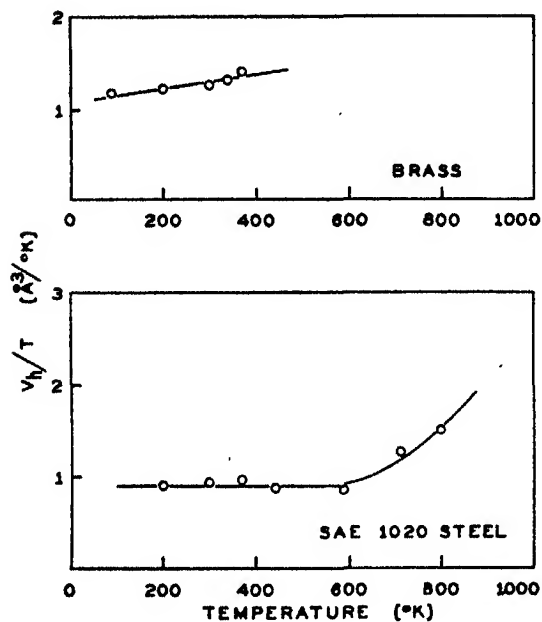


FIGURE 3

Curves showing V_h/T versus temperature relationship.

Thus, one is led to conclude that with the value of the constant and the compressibility known, the value of V_h is calculable from Eq. (9). However, if one uses the known compressibilities of steel and brass one obtains for the volume, V_h , a value which is unreasonably small. One is led, therefore, to the conclusion that the material being compressed or rarefied in metals is not the system of whole atoms, but the system of positive kernels; i.e., the atoms minus their conductance electrons.

Conductance electrons are capable of moving almost independently of short range motion of the kernel and consequently, the kernels can move relatively independent of the conductance electrons with restrictions determined largely by the proximity of the neighboring nuclei and the accumulation of charge. The conductance electrons then form a sort of continuum, inside of which the positively charged kernels fluctuate in density. Fluctuations in density of the kernels are known to be responsible for the thermal scattering of x-rays⁶ and a study of this phenomenon provides additional evidence that density variations are possible. The increase in electrical conductance with decreasing temperature is also

associated with a decreasing fluctuation of the density of the system of kernels thus increasing the mean free path of the conducting electrons.

In 1937 Ewell and Eyring⁷ in their study of liquids found that for flow, metals required an apparent hole about $1/18$ the size of the molecule as compared with about $1/7$ the size for non-metals and from this they concluded that the kernels must be flowing relatively independent of their conductance electrons. The size of the hole necessary for metals was found to be about $1/6$ – $1/7$ the size of the kernel, the same proportionate part of the volume previously found for non-metals.

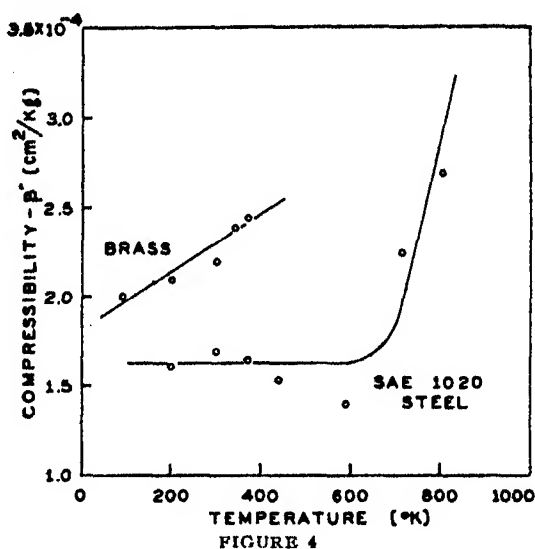


FIGURE 4
Coefficient of compressibility β^* versus temperature for brass and SAE 1020 steel.

One is thus led to interpret the compressibility factor in equation (9) as the value for the compression of a system of kernels in a sea of conductance electrons. Using the values of V_h calculated from experiment,⁸ the compressibility factors (denoted β^* for the system of kernels) determined from equation (9) are shown in figure 4 for the range of temperatures investigated. The results show that the kernel compressibility is about one thousand times that for the bulk metal and as an approximation one may write

$$\beta^* \approx \beta \times 10^3$$

A value of β^* so much greater than the bulk β^* (which accounts for the specific heat of metals in the Debye theory) indicates that flow occurs at special imperfect regions which comprise only a small part of all the

degrees of freedom. Rewriting equation (8), replacing β by β^* and V by V_h , one obtains

$$\frac{V_h(1/\beta^*)}{kT} = \frac{\Delta F^*}{kT} = \text{const.} \quad (10)$$

The question naturally arises as to the applications to be made of the theory. By looking at equation (6) it is clear that the most important factor affecting flow is the volume of the hole, V_h , since $\Delta F^*/T$ changes little with either temperature or the nature of the metal. Metals with a large value for V_h will flow exponentially easier than those with small values.

Non-metals are not separable into conductance electrons and kernels and therefore one understands why these substances do not have the ductility of metals. Preliminary investigation has shown that the factor $\Delta F^*/T$ is not independent of temperature and of substance for non-metals as it is for metals—a result one might expect.

The conductance electrons surrounding the kernels act as an external pressure on the kernels, decreasing the tendency for bonds to stay broken permanently, thus increasing the ductility. Hydrostatic pressure can be very effectively utilized to increase ductility as has been shown by Bridgman.⁸ This pressure, when applied to metals, supplements the pressure due to the conductance electrons in acting against permanent bond rupture in the flow process.

Any rigid imperfection or dislocation of the lattice which interferes with the lattice structure in such a way as to prevent the kernels from moving independently of their conductance electrons will constitute a barrier to flow. These imperfections, however they are introduced, will harden the pure metal. This point of view explains naturally the effect of the imperfections considered to be present by G. I. Taylor⁹ and others.

* This paper is based on a research program conducted at the University of Utah in cooperation with the Office of Naval Research, U. S. Navy Department.

† If the volume of the hole, V_h , is not formed by a fluctuation in density as the activated complex appears then V_h must be a hole already present in the metal as a non-equilibrium imperfection. In that case V and V_h could be entirely different with V (Eq. 8) even as much as a thousand times smaller than V_h and the compressibility β then approximating the compressibility of matter in bulk. We have neglected this possible alternative as less likely for metals than the identification of V with V_h .

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EVIDENCE FOR AGING AS A CONSEQUENCE OF GROWTH CESSATION*

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Communicated by T. N. Sonneborn, March 27, 1948

A previous investigation by Lansing¹ has indicated that a non-genic factor is active in aging of the rotifer. This factor has been shown to be transmissible, cumulative and reversible. The current report which is an extension of the initial experiments brings together new data which show that the aging factor becomes operative at a critical period in the life span. The period of growth cessation in the rotifer seems to coincide with expression of the aging factor as measured by accelerated aging.

The essential nature of the experimental method consisted of establishment by selection from isolation culture of series of generations of a parthenogenetic rotifer (*Philodina citrina*) with uniform parental age in all generations; such a series of generations was designated an "orthoclone." The isolation culture technique has been described previously.² It should be stressed that the experimental method involves no procedures which might alter the physiology of the rotifers. The longevity differences observed in various orthoclones are those apparently existing in nature and are only unravelled by the selection procedure.

The standardized environmental and genetic techniques yield longevity data for *Philodina citrina* which are remarkably consistent. Figure 1 shows diagrammatically the survival curves and egg production data for two closely related clones of rotifers. A wild stock group of 60 rotifers are contrasted with a similar group derived from eggs laid on the sixth day of life by the wild stock group. The curves for both longevity and egg production are virtually superimposed. The mean life span of the parent group was 25.6 ± 0.63 days while that of the offspring ($6F_1$) was 25.7 ± 0.55 . The mean number of eggs laid per day during adult life is approximately two eggs per day. It is interesting to note that the adult rate of egg production is reached on the sixth day of life. Egg production falls

off rapidly to an insignificant level after the fifteenth day of life. As indicated previously,¹ *Philodina citrina* appears to produce a maximum of 32 eggs during the life span of an individual.

With the culture method employed *Philodina citrina* hatches from the egg in one day, initiates laying of eggs on the fourth and fifth days of life, reaches adult size on the sixth day and begins to show senile changes on approximately the fifteenth day of life.

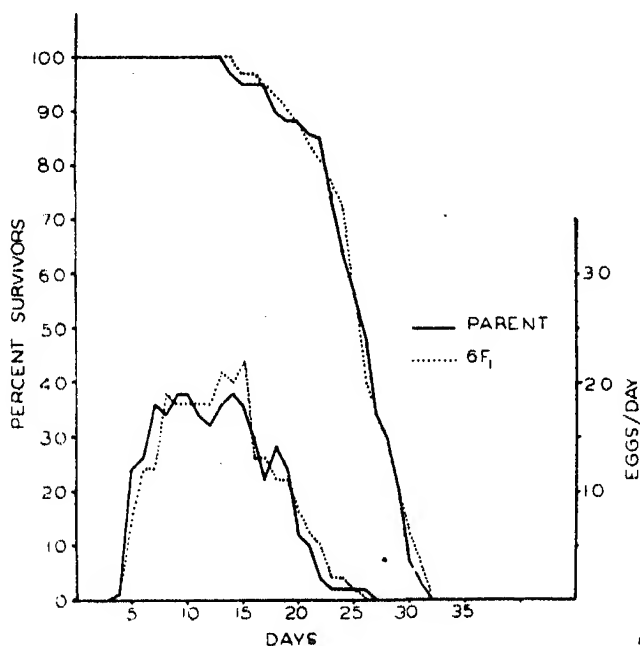


FIGURE 1

Survival and egg production curves for two related groups of *Philodina citrina*.

- Measurements of body lengths were made with a micrometer ocular to determine the time at which maximum size is reached. It is difficult to obtain accurate data by this procedure since *Philodina* varies its length during movement. These measurements were made while the rotifers were fully extended in creeping along the bottom surface of the pyrex depression slides. The results of these measurements are graphically illustrated in figure 2 and represent the means of ten measurements for each day of life studied. *Philodina citrina* apparently reaches maximum length between the fifth and sixth days of life. This time coincides with that of initiation of the adult rate of egg production.

The preceding investigation¹ showed that the longevity of successive

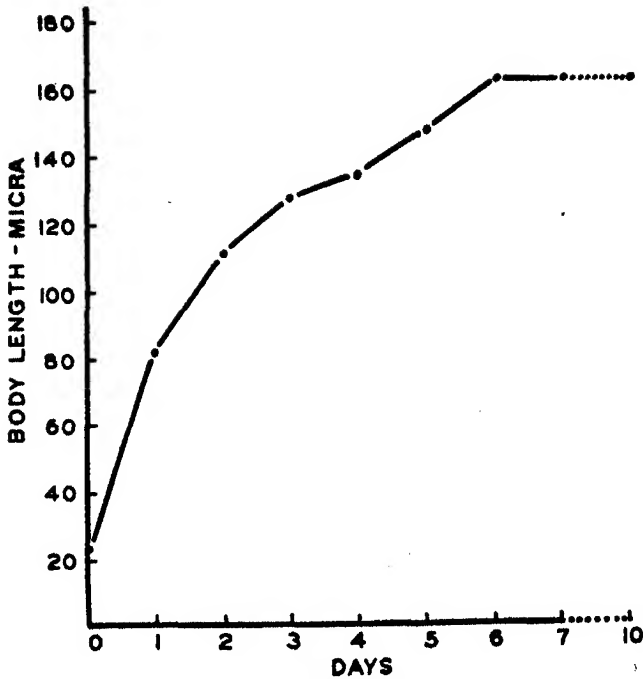


FIGURE 2

Graph showing changes in length of the rotifer in early life. Maximum length appears to be reached between the fifth and sixth days of life.

generations in old (16- and 17-day) orthoclones decreased progressively and that such old orthoclones invariably died out. Moreover, a similar pattern of events held true for eleven- and eight-day orthoclones. The latter result, illustrated graphically in figure 3, was particularly striking, for an eight-day old rotifer is a vigorous young adult animal. Since it was shown that orthoclones derived from adolescent (5-day old) rotifers manifest a progressive increase in longevity rather than a decrease, it seemed apparent that the aging factor must make its appearance or express itself early in life at some time between adolescence and early maturity. Six- and seven-day orthoclones were traced in detail in order to determine whether the factor appears abruptly or gradually between adolescence and early adult life.

Figure 4 contains the essential data of the 7-day orthoclone experiment. Survival curves for each generation in the orthoclone are shown in relation to one another. The pattern of events in the seven-day orthoclone parallels that of older orthoclones. The middle portion of the rectangular survival curve found in the first few generations tends to collapse as more animals

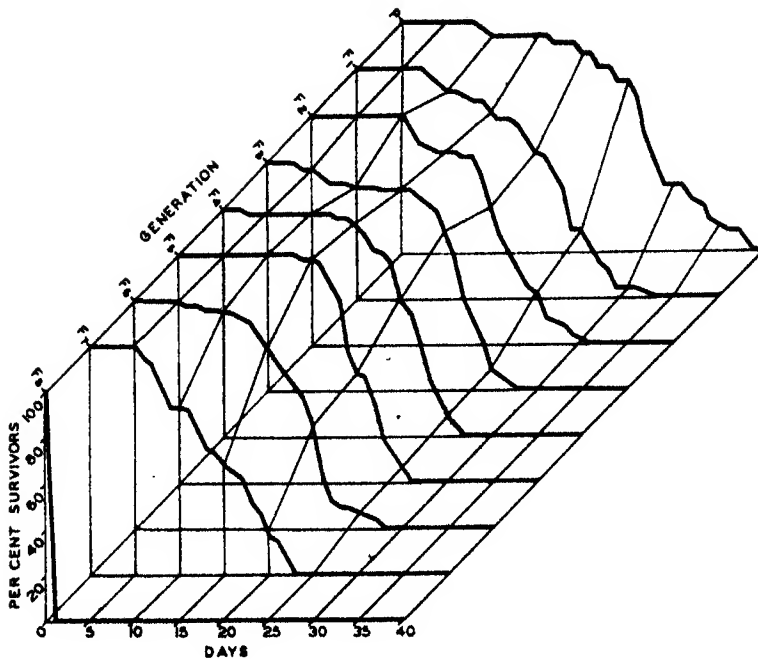


FIGURE 3

Three-dimensional graph of survival curves in successive generations of an 8-day orthoclone.

die at an early age. The $7F_6$ and succeeding generations show a fairly uniform mortality rate with little change in maximum life span. The $7F_{12}$ exhibits a distinct drop in mean life span and this is furthered through the $7F_{13}$ which yielded a mean life span of 6.5 ± 0.19 days. The $7F_{16}$ was the last generation that could be obtained since no eggs were laid by these animals at any time during their short life spans.

The six-day orthoclone (Fig. 5) exhibits a pattern of change essentially like that of the 7-day orthoclone. Here, again, the middle portion of the survival curve collapses, no further marked changes occur until the fourteenth generation. The orthoclone died out for lack of eggs in the seventeenth generation which yielded a mean life span of 7.7 ± 0.27 days.

It appears, then, that the aging factor expresses itself at some time between the fifth and sixth day of life. The time cannot be defined more clearly with the present technique since observations are made at 24-hour intervals.

The fact that the period between the fifth and sixth days of life in *Phlo-dina citrina* marks the transition is significant when related to appearance

of the aging factor. Certainly the view that aging begins in the fertilized ovum becomes untenable. These experiments to the contrary suggest that aging is a direct sequel to growth cessation. Apparently, at any time during active growth the organism is at least potentially immortal. It is only with growth cessation that the capacity for aging develops.

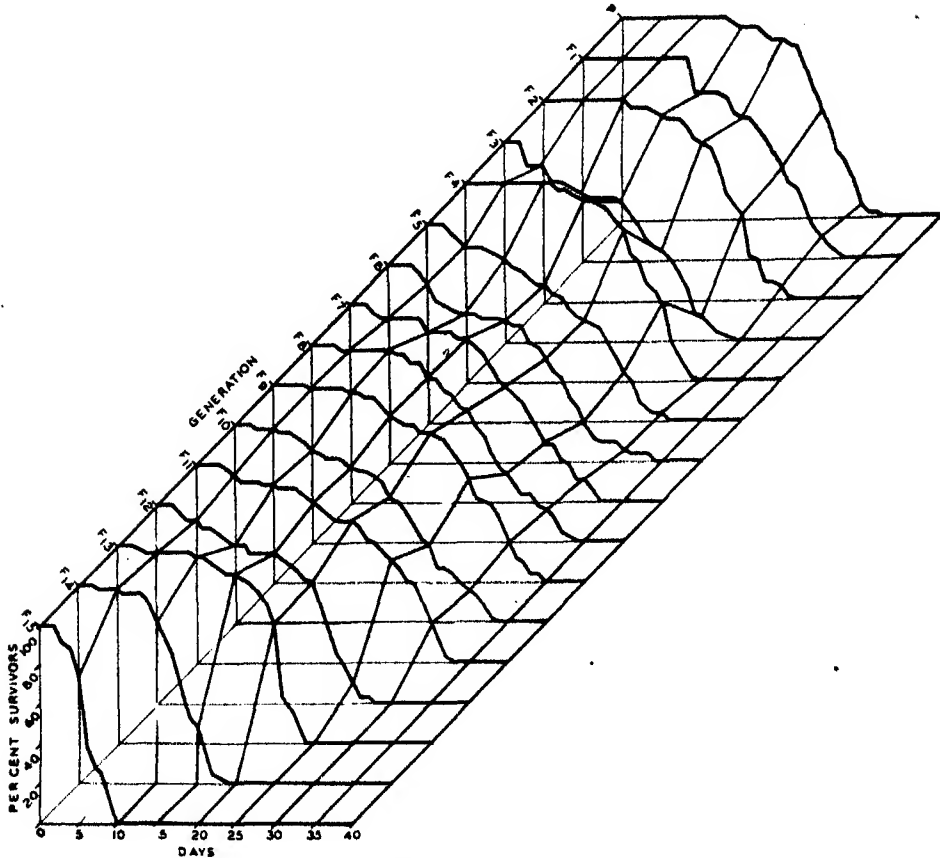


FIGURE 4

Three-dimensional graph showing relation between successive generations of a 7-day orthoclone.

Examination of figure 6 indicates that there is an orderly relation between longevity in generations of an orthoclone and age of the orthoclone. The curve that can be derived from the block diagram is essentially asymptotic. It seems, therefore, that the aging factor not only appears abruptly during the period of growth cessation but also increases with age after maturity.

Some critical observations remain to be made. The vertical limb of the asymptotic curve in figure 6 suggests that the longevity of a five-day orthoclone should be essentially infinite. This possibility is in accord with the observation that longevity of five-day orthoclones tends to increase. It remains to be demonstrated that the five-day orthoclone can

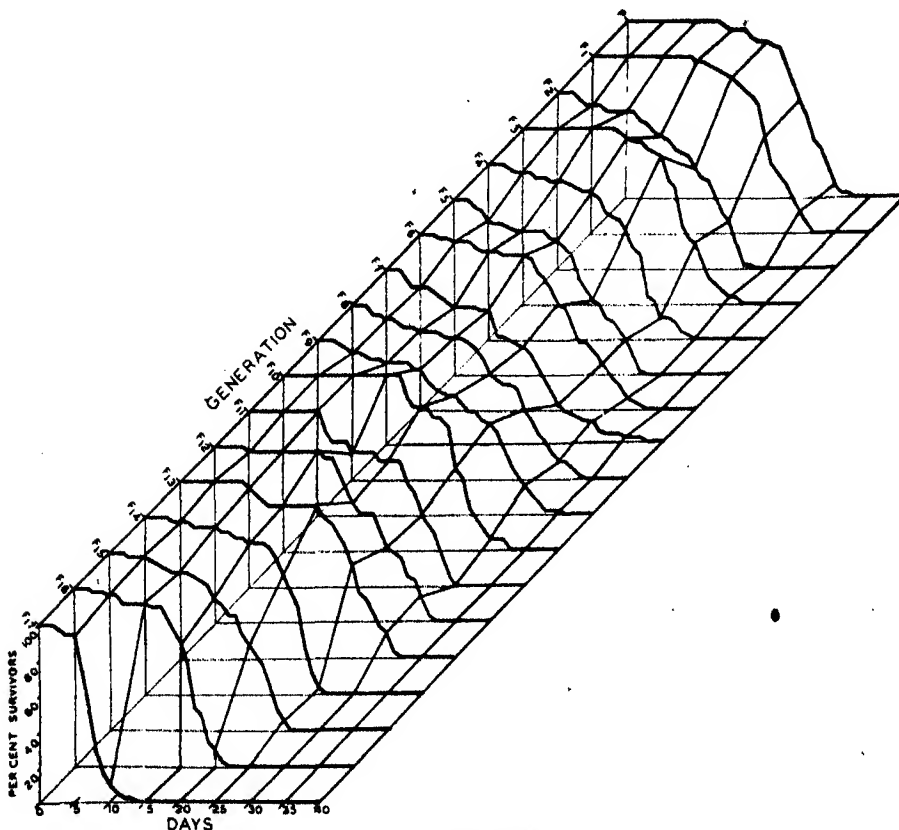


FIGURE 5

Three-dimensional graph showing relation between successive generations of a 6-day orthoclone.

be maintained indefinitely. Further, there as yet is no indication as to the maximum life span of rotifers in a five-day orthoclone.

The behavior of the aging factor and its relation to growth assumes additional significance when one examines in retrospect the meager literature on aging. Various experiments^{3, 4} on the effect of starvation on longevity contain the basic pattern that extension of the period of growth results in extension of the total life span. Sonneborn's⁵ experiments on

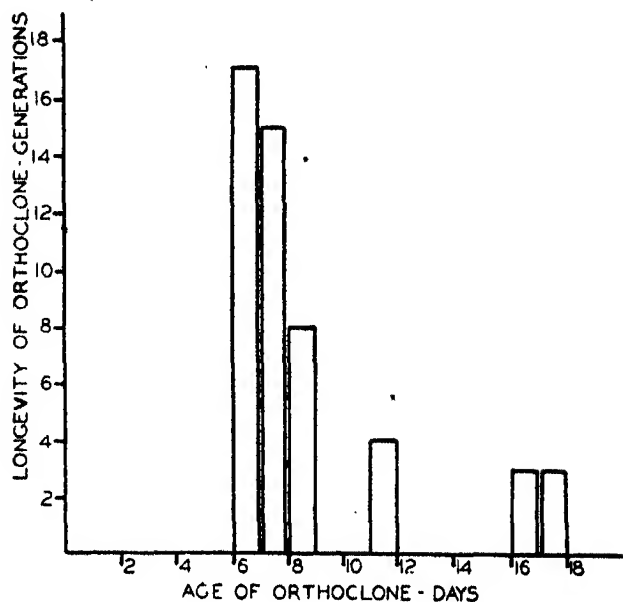


FIGURE 6

Recapitulation of relation between longevity of orthoclones and age of various orthoclones.

Stenostomum suggest that growing tissues do not age while non-growing tissues do age. In general it seems true that post-mitotic cells are the only ones which age, growing intermitotic cells do not show age changes.⁶

Summary.—It has been shown that aging of the rotifer involves a non-genetic factor which is transmissible, cumulative and reversible. New data have been obtained which indicate that the time of appearance of this aging factor coincides, within the limits of the experimental technique, with the time of cessation of growth.

There appears to be an inverse relation between the age of an adult orthoclone and longevity of such orthoclones.

* Aided by a grant from the U. S. Public Health Service.

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REFLECTION EFFECT IN ECLIPSING BINARIES FOR A POINT-SOURCE OF LIGHT

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Communicated by Harlow Shapley, May 17, 1948

Milne,¹ it is well known, was the first to formulate a darkening law for the reflected radiation and deduce the reflection effect in eclipsing binaries. Milne's phase law is, however, true to the order of the square of the radius (in units of the distance separating the two stars) of the reflecting component, as he considered the components to be so widely separated that the incident light constituted an approximately parallel beam.

Kopal² drew attention to the need for the evaluation of the reflection effect to higher order terms, as the fractional radii of the components in eclipsing binary systems are quantities of the order of one-tenth. In Kopal's words,

"In order to establish a closer picture of conditions to be met in actual binary systems..., the convergence of the cone of incident radiation should be considered as well as the finite angular size of the illuminating star. The respective exact equations belong to the class of those which are as easy to formulate as they are difficult to solve; so that relatively little is known about the behaviour of higher order reflection terms so far."

In the present paper, the finite radius of the reflecting star and the convergence of the incident beam are taken into account, but the illuminating star is treated as a luminous point.³ The phase-function has been found correct to the fourth power of the stellar radius (in units of the distance between the two stars). The results should be applicable to supergiant components with early-type companions in eclipsing systems like ζ Aurigae or VV Cephei. The present investigation is only a first step towards a complete solution, and it is hoped that the finite radius and the darkening of the illuminating star will be considered in a future communication.

To simplify the treatment, a cosine law of reflection has been assumed, analogous to Lambert's law in planetary photometry. The results, it

may be noted, would not be greatly vitiated by this assumption, as Kopal has shown by Fourier expansion that the simple cosine law does not deviate much from the more accurate phase laws.⁴ The approximation fails at the edge where, according to the cosine law, the intensity should vanish. Near the edge, however, the reflected radiation is so feeble in intensity that it contributes little to the total reflected light.⁵

We have taken as the unit of length the distance between the centers of gravity of the two stars, and have treated the reflecting star as spherical. This is valid to our order of approximation (fourth power of stellar radius), as the distortion is of the order of the cube of the radius and would therefore introduce terms of the order of the fifth and higher powers of the radius, in the reflected light.⁶

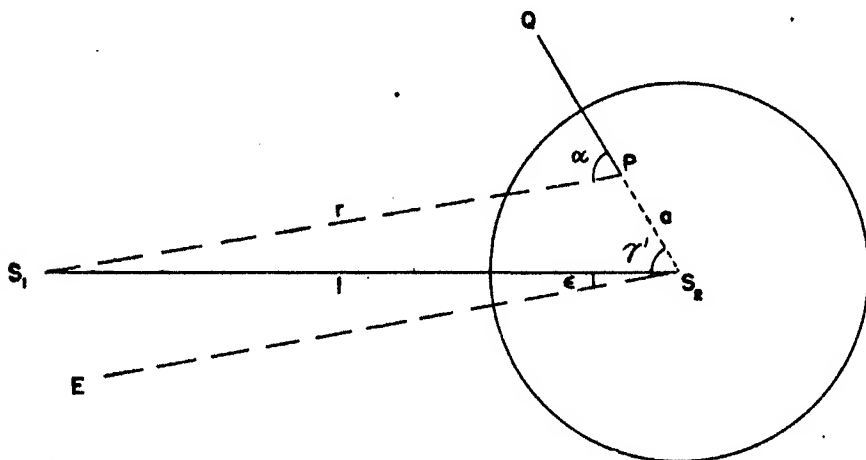


FIGURE 1

In figure 1, S_1 is the illuminating star, regarded as a point source of light. The illuminated star S_2 is of radius a , and the distance $S_1S_2 = 1$. S_2E is the direction of the observer. P is any point on the surface of S_2 , and $S_1P = r$. Let the phase-angle $S_1S_2E = \epsilon$, the angle of foreshortening $PS_2E = \gamma$, the angle of incidence $S_1PQ = \alpha$, and the angle $S_1S_2P = \gamma'$.

We have, by projection on S_2Q ,

$$\cos \gamma' = a + r \cos \alpha, \quad (1)$$

which gives

$$\begin{aligned} \cos \alpha &= \frac{\cos \gamma' - a}{r} \\ &= (\mu - a)(1 + P_1a + P_2a^2 + \dots) \\ &= \mu - (1 - \mu^2)a - \frac{3}{2}\mu(1 - \mu^2)a^2 \dots, \end{aligned} \quad (2)$$

where $\mu = \cos \gamma'$, and P_1, P_2, \dots are Legendre functions of μ .

We shall retain, as already mentioned, terms up to a^4 .

We express the position of P by spherical polar coordinates (Fig. 2) on the sphere S_2 .

N is the pole of S_1E . Let $NP = \eta$, and the angle $PNS_1 = \phi'$.

The element on the surface of S_2 ,

$$d\sigma = a^2 \sin \eta \, d\eta \, d\phi'. \quad (3)$$

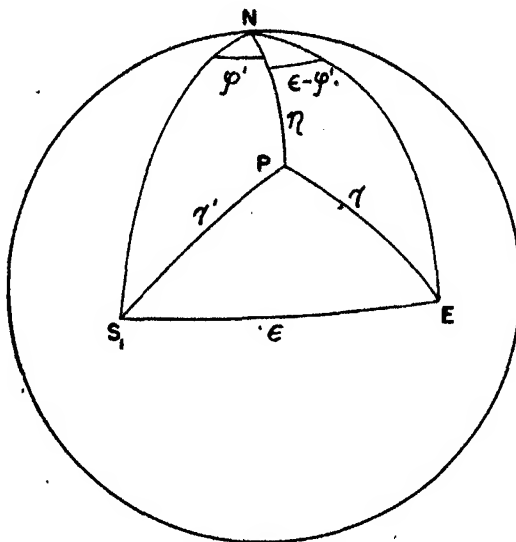


FIGURE 2

Also, from spherical triangles, NPS_1 and NPE , we have

$$\mu = \cos \gamma' = \sin \eta \cos \phi', \quad (4)$$

and

$$\cos \gamma = \sin \eta \cos (\epsilon - \phi'). \quad (5)$$

We shall assume, as already stated, that the reflected radiation is uniformly distributed in intensity according to the cosine law

$$I = S \cos \alpha, \quad (6)$$

where πS is the flux per unit area normal to itself, and α is the angle of incidence with the surface normal.

Now, we have

$$\begin{aligned}\pi S &= \frac{L}{4\pi r^2} \\ &= \frac{L}{4\pi} \{1 + 2\mu a + (4\mu^2 - 1)a^2 \dots\},\end{aligned}\quad (7)$$

where L is the luminosity of the illuminating star (S_1).

From equations (2), (6) and (7), we have

$$I = \frac{L}{4\pi^2} \left\{ \mu + (3\mu^2 - 1)a + \left(\frac{15}{2}\mu^3 - \frac{9}{2}\mu \right) a^2 \dots \right\}. \quad (8)$$

From equations (3), (5) and (8), we have that the amount of reflected light received by the observer is given by

$$\begin{aligned}\int I \cos \gamma \, d\sigma &= \frac{L}{4\pi^2} a^2 \int_{\epsilon-\pi/2}^{\cos^{-1} a} \int_{\sin^{-1}(a \sec \phi')}^{\pi - \sin^{-1}(a \sec \phi')} \left\{ \mu + (3\mu^2 - 1)a + \right. \\ &\quad \left. \left(\frac{15}{2}\mu^3 - \frac{9}{2}\mu \right) a^2 \right\} \cos(\epsilon - \phi') \sin^2 \eta \, d\phi' \, d\eta. \quad (9)\end{aligned}$$

The limits for η are derived from the consideration of the section of the plane $\phi' = \text{constant}$ by the base of the enveloping cone. This is given by $\sin \eta = a \sec \phi'$. The limits for ϕ' indicate that we are considering at first the partial phase, that is, when a part only of the illuminated portion of S_2 is visible to the observer. The integration in the right-hand side of the equation (9) is to be performed with respect to η first.

We substitute for μ from (4) in (9) and integrate (9) term by term, evaluating each partial integral at first in closed form and then expanding the latter in ascending powers of a . Denoting the partial integrals by I_1 , I_2 and I_3 , we arrive at the following values:

$$\begin{aligned}I_1 &= \int_{\epsilon-\pi/2}^{\cos^{-1} a} \int_{\sin^{-1}(a \sec \phi')}^{\pi - \sin^{-1}(a \sec \phi')} \cos \phi' \cos(\epsilon - \phi') \sin^2 \eta \, d\phi' \, d\eta \\ &= \frac{2}{3} \{(\pi - \epsilon) \cos \epsilon + \sin \epsilon\} - a^2 \sin \epsilon\end{aligned}\quad (10)$$

$$\begin{aligned}I_2 &= a \int_{\epsilon-\pi/2}^{\cos^{-1} a} \int_{\sin^{-1}(a \sec \phi')}^{\pi - \sin^{-1}(a \sec \phi')} (3 \sin^2 \eta \cos^2 \phi' - 1) \cos(\epsilon - \\ &\quad \phi') \sin^2 \eta \, d\phi' \, d\eta = \frac{3\pi}{8} a (1 + 2 \cos \epsilon + \cos^2 \epsilon) - \frac{\pi}{2} a (1 + \\ &\quad \cos \epsilon) + 2a^2 \sin \epsilon.\end{aligned}\quad (11)$$

$$\begin{aligned}I_3 &= a^2 \int_{\epsilon-\pi/2}^{\cos^{-1} a} \int_{\sin^{-1}(a \sec \phi')}^{\pi - \sin^{-1}(a \sec \phi')} \left(\frac{15}{2} \sin^3 \eta \cos^3 \phi' - \frac{9}{2} \sin \eta \cos \phi' \right) \\ &\quad \cos(\epsilon - \phi') \sin^2 \eta \, d\phi' \, d\eta = -a^2 \sin^3 \epsilon.\end{aligned}\quad (12)$$

Hence we have for the reflection effect for the partial phase

$$L_p = \frac{L}{4\pi^2} a^3 \left[\frac{2}{3} \{ (\pi - \epsilon) \cos \epsilon + \sin \epsilon \} + \frac{\pi}{8} a (3 \cos^2 \epsilon + 2 \cos \epsilon - 1) + a^2 \sin \epsilon \cos^2 \epsilon \right]. \quad (13)$$

We shall now consider the complete phase, that is, when the illuminated portion of S_2 is fully visible to the observer. This will be so long as $\epsilon < \sin^{-1} a$. The limits for ϕ' in this case will be $\cos^{-1} a$ and $-\cos^{-1} a$.

We have for the complete phase

$$I_1 = \frac{2}{3} \pi \cos \epsilon,$$

$$I_2 = \frac{3}{2} \pi a \cos \epsilon - \pi a \cos \epsilon,$$

and

$$I_3 = 3\pi a^2 \cos \epsilon - 3\pi a^2 \cos \epsilon.$$

Hence we have the reflection effect for the complete phase

$$L_f = \frac{L}{4\pi^2} a^2 \left(\frac{2}{3} \pi \cos \epsilon + \frac{\pi}{2} a \cos \epsilon + 0 \cdot a^2 \right). \quad (14)$$

It should be noted that when $\pi - \sin^{-1} a < \epsilon < \pi$, the star S_2 will be in eclipse phase, that is, no reflected light will be received by the observer.

In a letter to Kopal,³ Russell mentioned the following useful check which the formulae for the reflection effect should satisfy. The portion of the incident light from S_1 intercepted by S_2 lies within the solid angle

$$\omega = 2\pi(1 - \cos s), \quad (15)$$

where s is the angle which the tangent from the point S_1 to the star S_2 makes with the line $S_1 S_2$ (see Fig. 1).

Now, we have

$$\begin{aligned} \cos s &= \sqrt{1 - a^2} \\ &= 1 - \frac{1}{2}a^2 - \frac{1}{8}a^4 \dots, \end{aligned} \quad (16)$$

to our order of approximation.

From equations (15) and (16) we have that the proportion of the incident light of star S_1 intercepted by S_2 is

$$\frac{\omega}{4\pi} = \frac{1}{4}a^2 + \frac{1}{16}a^4 \dots \quad (17)$$

As all incident light is reflected back, the proportion of the incident

light received as reflected light by the observer should also satisfy equation (17). We verify below that our formulae satisfy Russell's check.

We evaluate the proportion of reflected light received by the observer in three steps as follows:

(1) Partial phase ($a < \epsilon < \pi - a$).

$$\begin{aligned} \frac{2\pi}{L} \int_a^{\pi-a} L_r \sin \epsilon \, d\epsilon &= \frac{a^2}{2\pi} \int_a^{\pi-a} \left[\frac{2}{3} \{ (\pi - \epsilon) \cos \epsilon + \sin \epsilon \} + \right. \\ &\quad \left. \frac{\pi}{8} a (3 \cos^2 \epsilon + 2 \cos \epsilon - 1) + a^2 \sin \epsilon \cos^2 \epsilon \right] \sin \epsilon \, d\epsilon \\ &= \frac{1}{4} a^2 - \frac{5}{48} a^4. \end{aligned}$$

(2) Complete phase ($0 < \epsilon < a$).

$$\begin{aligned} \frac{2\pi}{L} \int_0^a L_r \sin \epsilon \, d\epsilon &= \frac{a^2}{2\pi} \int_0^a \left(\frac{2}{3} \pi + \frac{\pi}{2} a \right) \cos \epsilon \sin \epsilon \, d\epsilon \\ &= \frac{1}{6} a^4. \end{aligned}$$

(3) Eclipse phase ($\pi - a < \epsilon < \pi$).

There is obviously no contribution to the reflected light in this phase.

Hence the proportion of the incident light received as reflected light by the observer is

$$\frac{1}{4} a^2 + \frac{1}{16} a^4 \dots \quad (17)$$

Our formulae for the reflection effect therefore satisfy Russell's check to the adopted order of approximation.

The author desires to offer his sincere thanks to Dr. Zdeněk Kopal¹ for suggesting the problem and for considerable helpful criticism during the course of the work. The author's best thanks are also due to Professor Henry Norris Russell for illuminating discussion, to Professor Harlow Shapley for the very valuable privilege of working at the Harvard College Observatory, and to Mr. Arthur Hoag for kindly drawing the figures.

Summary.—The reflection effect for a point illuminating source has been evaluated, correct to the fourth power of the ratio of the radius of the illuminating star to the distance between the two stars, on the hypothesis of a cosine law of reflection.

¹ *M. N.*, 87, 43 (1926).

² *An Introduction to the Study of Eclipsing Variables*, 1946, p. 155.

³ Dr. Kopal in his *Eclipsing Variables* (*loc. cit.*, p. 155) has given an outline of a solution initiated by Takeda (*Kyoto Mem.*, A17, 197 (1934)). But, as Prof. Russell pointed

out in a letter to Dr. Kopal, the upper limit of $\pi/2$ in Dr. Kopal's integral of the reflection effect extends it beyond the convergent cone. At Dr. Kopal's suggestion, the author has rigorously evaluated, in this paper, the integral with the proper limits.

⁴ *Loc. cit.*, p. 154.

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⁶ Kopal, *Eclipsing Variables*, p. 164.

THE STIMULATORY ACTION OF CERTAIN FRACTIONS FROM BACTERIA AND YEAST ON THE FORMATION OF A BACTERIAL VIRUS

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Recent experiments have shown that the formation of bacterial viruses and the response of the host cells depend upon the composition of the culture medium. Wahl¹ has shown that some coli bacteriophages may be formed in synthetic medium without lysis of the host provided a certain concentration of thiamin and calcium is added to the medium. Raising the concentration of thiamin results in lysis of the host although no more virus is formed than without lysis. Experiments from this laboratory² have shown that *Staphylococcus muscae* phage can also be released in synthetic medium without lysis of the host. The addition of a substance present in veal infusion results in lysis of the host in the latter system. Fowler and Cohen,³ using a coli phage, have been able to increase the virus yield per cell by varying the composition of the medium. Maurer and Woolley⁴ have reported that the addition of apple pectin to synthetic medium permits the release of *E. coli* phage without cellular lysis.

It has now been found that a fraction from yeast or from the bacterial cell stimulates the formation of a bacterial virus. This fraction has a high concentration of ribonucleoprotein and has been referred to as the ribonucleoprotein fraction by other workers.⁵ The phage-stimulating property parallels the ribonucleoprotein fraction during purification. Splitting the ribonucleoprotein into free nucleic acid and protein causes complete loss of the phage-promoting activity. In view of these results, our working hypothesis is that the active substance is a ribonucleoprotein until experiments may prove otherwise.

A ribonucleoprotein fraction from yeast has recently been shown to stimulate the formation of adaptive enzymes in yeast.⁶ This nucleoprotein fraction has also been found to accelerate the formation of a bacterial

virus. These results, together with a comparison of adaptive enzyme formation and bacterial virus formation, will be described in this paper.

Experimental.—The *Staphylococcus muscae* phage system described previously was used in all the experiments.⁷ The cells and virus were grown as reported earlier.² Bacteria and phage were determined as in earlier experiments.⁷ The synthetic medium was the same as that de-

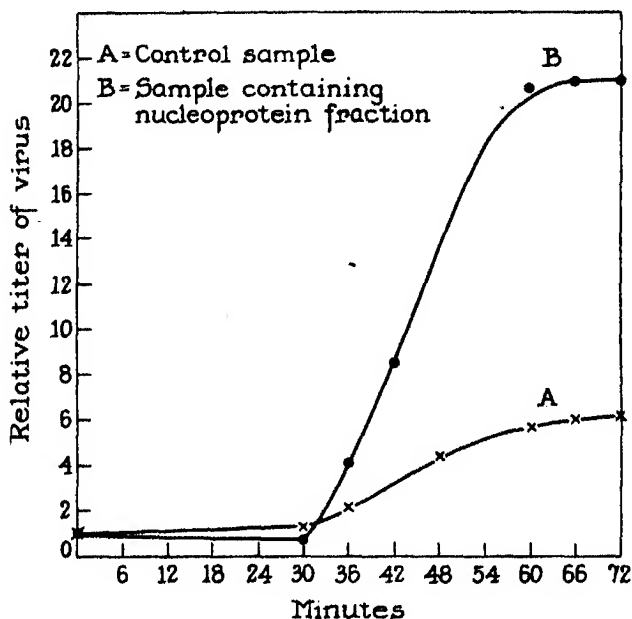


FIGURE 1

The effect of the bacterial ribonucleoprotein fraction on the formation of bacterial viruses. The bacterial cells were washed off a 20-hour old veal infusion agar slant and prepared as described under Methods. Two tubes of synthetic medium containing 2.0 mg. of hydrolyzed casein were inoculated with 5.8×10^7 cells per ml. Tube A was the control and tube B received 0.1 ml. of the ribonucleoprotein fraction containing 0.7 γ of nitrogen per ml. After 1 hour of incubation both tubes contained 6.2×10^7 cells per ml. To each tube 0.1 ml. of a virus solution was then added to give a final titer of 4.8×10^7 particles per ml. The tubes were shaken 18 minutes and then diluted 1:2000 in their respective media. The control tube showed 43% of the virus adsorbed and the tube containing the nucleoprotein showed 40.7% of the virus adsorbed. Samples were taken from the diluted tubes for virus assay at various times.

scribed previously.² One-step growth curves were carried out according to Delbrück and Luria.⁸

Most of the nucleoprotein fractions were prepared from bacterial cells first ground with powdered glass and then extracted with water. After centrifugation the supernatant fluid was adjusted to pH 4.0 with 10%

acetic acid and 1 volume of alcohol added. The precipitate was collected, resuspended in water and adjusted to pH 6.3 with 0.5 *M* NaHCO₃. This solution was adjusted to pH 3.7 with 10% acetic acid. The precipitate was collected and the acid precipitation repeated. The yeast ribonucleo-protein fraction was prepared from fresh baker's yeast in a similar manner, except that the crude water extract was dialyzed against water for 10 hours before the alcohol fractionation. All operations were carried out at 5°.

Results.—The addition of the nucleoprotein fraction does not increase the per cent adsorption of the virus to the cell nor does it decrease the minimum latent period (Experimental Procedure in figure 1). It does increase the yield of virus per cell. As shown in figure 1, for the first 30 minutes the phage count, which represents the unadsorbed phage and the

TABLE 1

THE EFFECT OF VARYING CONCENTRATIONS OF THE BACTERIAL NUCLEOPROTEIN FRACTION ON THE FORMATION OF THE BACTERIAL VIRUS

The same conditions were used as in figure 1. The values below represent the maximum titer at the end of a one-step growth curve. The initial titer was 2.1×10^4 plaque counts per ml.

SAMPLE	γ N OF RIBONUCLEOPROTEIN FRACTION ADDED PER ML.	% ADSORPTION OF VIRUS TO CELL	FINAL PLAQUE COUNTS PER ML.
1	..	40.2	1.1×10^5
2	0.22	42.1	2.1×10^5
3	0.44	38.6	3.1×10^5
4	0.88	41.3	5.3×10^5
5	1.6	39.1	5.6×10^5

adsorbed phage, remains constant. This period is called the minimum latent period.⁸ At the end of 30 minutes, the phage titer goes up for 30 minutes as the virus particles are released by the infected cells. Due to the high dilution step used in the one-step growth curve, the phage titer no longer increases after the initially infected bacteria have released their phage, since there is no readsorption of the released virus particles to new cells. The average yield of viruses per cell may be calculated by the equation⁸ below.

$$\text{Average burst size} = \frac{\text{final virus} - \text{initially unadsorbed virus}}{\text{initial virus} - \text{initially unadsorbed virus}}$$

In the experiment shown in figure 1 the control tube formed an average of 7 viruses per cell and the tube containing the nucleoprotein fraction, 31 virus particles per cell. In the hundreds of tests that have been run, the nucleoprotein fraction from yeast or bacteria has increased the relative phage titer from 2 to 30 times over the control. The average stimulation

is generally fourfold. It should be noticed that these tests are run with cells in the resting phase. Cells in the log phase form more phage than those in the resting phase and under these conditions the nucleoprotein fraction has only a slight effect, increasing the formation of phage not quite two times. It appears that this substance may be synthesized by rapidly growing cells. The experiment in table 1 shows the effect of varying concentrations of the nucleoprotein fraction on the formation of the virus.

The ultra-violet absorption curve of a highly purified fraction of the yeast ribonucleoprotein fraction shows a sharp maximum at 2600 A. U. and a minimum at 2450 A. The minimum at 2450 A is characteristic of nucleoproteins rather than free nucleic acid. The minimum of free nucleic acid is at 2300 A.⁹ Splitting the nucleoprotein into free nucleic acid and protein causes a complete loss of phage-promoting activity. In the present state of purity, the phage-stimulating activity of the ribonucleoprotein fraction is not destroyed on incubation with trypsin, chymotrypsin, pepsin, ribonuclease or desoxynuclease. It is, of course, not dialyzable. It is not precipitated by cold trichloroacetic or metaphosphoric acid, is very poorly precipitated by ammonium sulfate, and is maximally precipitated by acid at around pH 3.7. It is not spun down by centrifuging 1 hour at 24,000 r. p. m. at pH 6.8 in a Bauer-Pickels air-driven centrifuge. The best bacterial preparation contained 15.3% nitrogen and 2.6% phosphorus. The nucleic acid is all of the ribose type.

Ribonucleoprotein fractions prepared from calf thymus, pancreas and liver are inactive. The virus, which contains mostly if not all desoxynucleic acid, inactivated by mild heating or acid, has no phage-promoting activity under our conditions. The conditions used to inactivate the phage do not inactivate the ribonucleoprotein factor. Tobacco mosaic virus, which is a ribonucleoprotein, shows no phage stimulating activity in our system.

Several months ago in a short abstract Reiner and Spiegelman announced the isolation from yeast of a ribonucleoprotein fraction which stimulated the formation of adaptive enzymes in yeast.⁶ In view of the great similarity between their substance and the substance being studied in this laboratory, their adaptive enzyme fraction was tried in our phage system. Table 2 shows that it acted exactly like the fraction isolated by us and had approximately the same activity per mg. of nitrogen as our fraction. All three purified preparations sent to us by Dr. J. Reiner were active in our system. Our compound is not active in the adaptive enzyme system. This is not surprising since their substance must be isolated in the presence of galactose.

Discussion.—The experiments reported in this paper show that the addition of a fraction, from the host bacterium or from yeast, containing ribonucleoprotein increases the virus yield per cell of infected bacteria.

The ribonucleoprotein fraction isolated from yeast by Reiner and Spiegelman⁹ which stimulates adaptive enzyme formation is very similar to the substance isolated in this laboratory. Indeed, it may replace it in stimulating phage formation.

It is difficult at the present time to understand the mechanism involved in the stimulation of the formation of a phage containing desoxynucleic acid by the ribonucleoprotein fraction. A theory has been proposed by Spiegelman¹⁰ to account for the action of his ribonucleoprotein fraction in enzyme formation. We have no evidence for or against his theory at the present time.

It appears to us that two possibilities exist: (1) the factor acts as a precursor-like or nutrient substance or (2) it effects some reaction necessary in virus synthesis. As a working hypothesis, the latter theory is being tested. From the work of Caspersson¹¹ and Brachet¹² it appears that

TABLE 2
THE EFFECT OF THE YEAST ADAPTIVE ENZYME FACTOR ON THE FORMATION OF THE BACTERIAL VIRUS

The same conditions were used as described in figure 1. The values below represent the maximum virus titer at the end of a one-step growth curve. The amounts of nitrogen used represent that concentration which gave 50% stimulation. The initial titer was 3.1×10^4 plaque counts per ml. The adsorption was approximately 40% in all samples.

SAMPLE	ADDITIONS	FINAL PLAQUE COUNTS PER ML.
1	1.1×10^5
2	Ribonucleoprotein fraction isolated in this laboratory (1.1γ N per ml.)	2.7×10^5
3	Enzyme stimulatory factor (0.81γ N per ml.)	3.1×10^5

ribonucleic acid is somehow concerned in protein formation. This, together with the observation that the ribonucleoprotein fraction also increases enzyme formation, makes it a likely hypothesis that the fraction is somehow accelerating protein synthesis. Chemical determinations of cells grown in the presence of this fraction have so far shown no differences over control cells, and cells, even in the lag phase, do not multiply faster in the presence of the compound.

Another interesting point brought out by these experiments is the similarity between adaptive enzyme formation and bacterial virus formation. This similarity was pointed out by Northrop¹³ and, although this view was not very popular, it should be reexamined in the light of new experimental evidence. An adaptive enzyme is formed by cells when grown in the presence of its substrate just as cells form phage when grown in the presence of phage. Before the production of either adaptive

enzyme¹⁴ or virus,⁸ there is a lag period. Both enzyme¹⁴ and virus¹⁸ may be formed in non-viable cells. Cells which have the ability to multiply rapidly appear to form adaptive enzymes¹⁶ and viruses¹⁷ better than old cells. A ribonucleoprotein fraction from yeast stimulates both virus and adaptive enzyme formation. One of the great differences that has existed between adaptive enzymes and viruses has been the destructive effect of the virus on the host. Even this difference, however, is not a very sharp one, since it has been found that the pathological response of the host to the virus may be modified by varying the medium.^{1, 2, 4}

Competitive interactions in the cell exist in the formation of both adaptive enzymes and bacterial viruses. Thus Spiegelman¹⁸ has shown that the formation of an adaptive enzyme in yeast may cause decreases in the other enzyme systems of the cell. In the formation of bacterial viruses in the *E. coli* system, Cohen¹⁹ has presented evidence that the formation of the virus prevents the synthesis of cellular constituents. The very interesting observations of Monod²⁰ on the formation of adaptive enzymes in bacteria are important in this connection. On exposing a cell to two sugars simultaneously, only one adaptive enzyme was synthesized. A similar situation in phage formation has been found by Delbrück and Luria⁸ who infected a bacterium with two different viruses and found that only one multiplied. Thus in both adaptive enzyme formation and bacterial virus multiplication there may exist a mutual exclusion effect. Finally, there are the observations, with a few exceptions, that in bacteria, viruses¹⁹ and adaptive enzymes²⁰ both need a source of external nitrogen for their formation.

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VITAMIN K₈ AS AN INHIBITOR OF THE GROWTH OF FUNGI AND OF FERMENTATION BY YEAST*

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The present report is concerned with new biological observations on one of the vitamin K analogues, vitamin K₈, known chemically as 2-methyl-4-amino-1-naphthol hydrochloride. This compound which has been used with remarkable success as a water-soluble form of antihemorrhagic vitamin,¹ is characterized by low toxicity for animals, the LD₅₀ for mice being approximately 750 mg./Kg. when administered orally² or intraperitoneally.³ Discussion of the chemistry and pharmacology of vitamin K and related compounds and methods of laboratory synthesis of different analogues of the former have been published.^{4, 5, 6} Due to their inhibitory action on the formation of acid in saliva, both vitamins K₈ (2-methyl-1,4-naphthoquinone) and K₉ may find practical application in prevention of dental caries.^{7, 8} Vitamin K₉ and 2-methyl-1,4-naphthoquinone retard the growth of *Penicillium notatum*,⁹ of other fungi, and of yeasts.¹⁰ Vitamin K₈, however, appears not to have been studied for antifungal properties.

The vitamin K₈ (2-methyl-4-amino-1-naphthol hydrochloride) used in the following experiments was synthesized as previously described by one of us¹¹ with slight modifications to improve the yield.

Inhibition of Growth of Fungi.—*Penicillium notatum* and *Trichophyton mentagrophytes* were selected for study as representatives of non-pathogenic and pathogenic fungi, respectively. A heavy inoculum of spores of *P. notatum* was seeded into each of several mushroom spawn bottles containing a corn steep liquor and mineral salt culture solution to which different amounts of vitamin K₈ were added. The initial pH of all solutions was adjusted to 5.5. Each concentration was studied in duplicate or triplicate in each experiment. The experiment was repeated three times, always with the same result. Growth of the mold was inversely

related to the concentration of K_5 : a concentration of 100 mg./L. (100 p. p. m.) suppressed all growth. This is shown in figure 1 which is a reproduction of a photograph taken two weeks after inoculation.

The effect of K_5 on *T. mentagrophytes* was studied using Sabouraud's medium and the well-known agar cup-plate technique. The Petri dishes used were 9 cm. in diameter: the central wells cut in the agar with a sterile



FIGURE 1

Growth of *Penicillium notatum* on a culture solution containing corn steep liquor, mineral salts, and different amounts of vitamin K_5 . The concentrations of K_5 are indicated in mg./L. at the bottom of each bottle. Photograph taken two weeks after inoculation. Growth was markedly retarded by a concentration of 50 mg./L. and was checked completely by a concentration of 100 mg./L.

cork borer were 1.1 cm. in diameter. A 1 per cent solution of K_5 checked growth of the fungus on the entire surface of the plates when placed in the central wells. A 0.1 per cent solution (1000 p. p. m.) produced zones that averaged 6.2–6.3 cm. in diameter. It seems probable that in broth cultures where the factor of diffusion of the comparatively large molecule through agar would be eliminated, lower concentrations might suffice

to prevent growth of this organism, since the compound completely checked growth of the fungus when incorporated in the nutrient agar in a concentration of 0.001 per cent.

Similarly vitamin K₃ was observed to be endowed with strong fungistatic

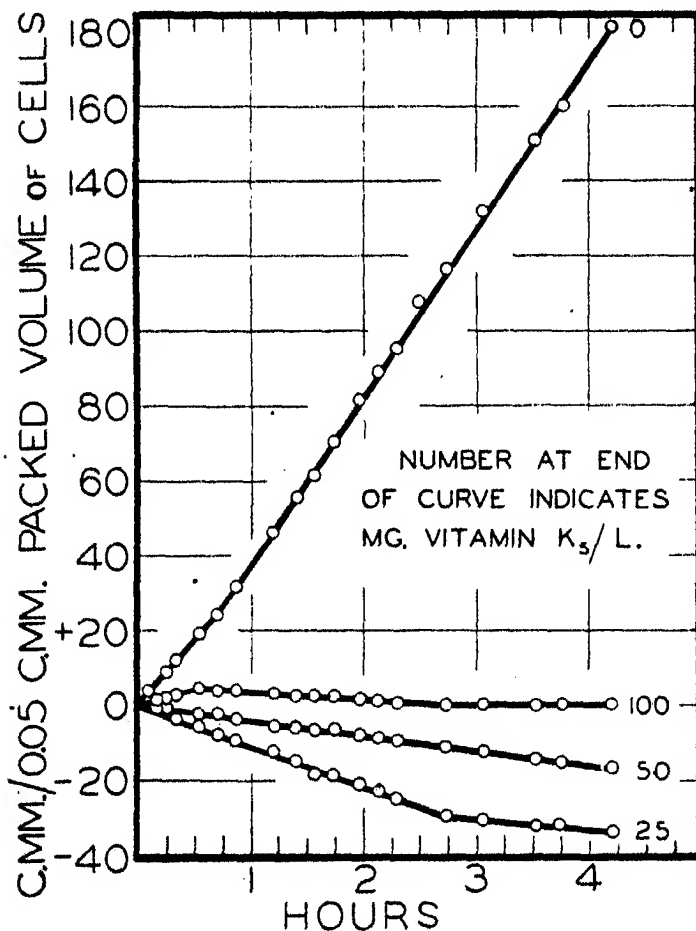


FIGURE 2

Change in gas pressure produced by yeast cultures in 10 per cent glucose with different concentrations of vitamin K₃.

activity when *Microsporum canis*, *M. audouinii* and the plant pathogen *Botrytis allii* were employed as test organisms.

Inhibition of Yeast.—The effect of different concentrations of vitamin K₃ on production of CO₂ by yeast (*Saccharomyces cerevisiae*) in 10 per cent

glucose was studied manometrically by means of the Warburg technique. All solutions were adjusted to pH 4.8. The averaged results of several experiments are shown in figure 2. Since the suspensions of yeast cells were placed in vessels with no KOH or other agent to absorb the gas that was liberated, an increase in pressure (curves with a positive slope) may be interpreted to mean that evolution of CO₂ exceeded consumption of O₂, and a decrease in pressure (curves with a negative slope) may be interpreted to indicate the opposite condition, i.e., consumption of O₂ exceeded evolution of CO₂ during the period of measurement.

The data plotted in figure 2 show that vitamin K₈ at a concentration of 25 mg./L. increased absorption of O₂ relative to evolution of CO₂. It is noteworthy that the points describe two straight lines rather than a curve with continuously changing slope. The sharp break in the curve suggests that the availability of a substrate on which rapid O₂ uptake depends was diminished until its concentration fell below a certain threshold, at which time another substrate must have come into play.

A similar but less pronounced excess of O₂ uptake relative to CO₂ evolution was apparent in the presence of 50 mg. K₈/L. At a concentration of 100 mg./L. the relative excess of O₂ uptake became apparent after an initial lag period of approximately one-half hour and continued for approximately two hours. Then the curve of slight negative slope reached the initial zero line where it remained throughout the duration of the experiment, due either to restoration of an RQ of 1 or to complete blocking of gas exchanges between the cells and the external medium.

The control curve shows that in the absence of vitamin K₈ a constant RQ greater than 1 was reached after approximately one hour and was maintained for the duration of the experiment.

The experimental results are reminiscent of the observation that appropriate concentrations of naphthoquinones or related compounds induce a transient increase in the rate of utilization of intracellular materials in algae, but hinder that of substances entering the cell from outside and thereby eventually disrupt metabolic activity.¹²

Vitamin K₈ at concentrations as high as 100 mg./L. did not inhibit the ability of the yeast to reduce colorless triphenyltetrazolium chloride to the red formazan, a reaction which is considered to depend upon dehydrogenase systems requiring coenzymes I and II.¹³ Conversely, solutions containing 25 mg. or more of K₈ per L. inhibited the indophenol oxidase system in yeast. Normally yeast cells promote rapid formation of indophenol-blue from a mixture of dimethyl-*p*-phenylene-diamine and thymol, but after having been exposed to solutions containing 25 to 100 mg. K₈/L., they not only tend to prevent formation of indophenol-blue but, if brought into the presence of the dye, they reduce it.

Discussion.—Our experimental results with vitamin K₈ are in accord

with those of Ball, *et al.*,¹⁴ who found that substituted naphthoquinones prevent the oxidation of *p*-phenylenediamine and interfere with the respiratory systems at a redox potential between those at which cytochrome *C* and cytochrome *B* operate. More specifically vitamin K₈ may be assumed to interfere with the component which Bach, *et al.*,¹⁵ consider necessary for the reduction of cytochrome *C* by cytochrome *B*, and which Slater¹⁶ tentatively identified as a haematin compound. It may be suggested that vitamin K₈ may act by the same mechanism that Schönberg and co-workers¹⁷ postulated for the various naphthoquinones, viz., by virtue of a high oxidation-reduction potential.

Because of its fungistatic activity and its low toxicity, vitamin K₈ may prove of value for the preservation of foods and beverages as well as for clinical treatment of dermatophytic fungi.

Summary.—Vitamin K₈ (2-methyl-4-amino-1-naphthol hydrochloride) at a concentration of 0.1 Gm./L. prevents growth of *Penicillium notatum* NRRL 1249.B4 on a rich culture medium. Growth of *Trichophyton mentagrophytes* was prevented by K₈ at a concentration of 10 Gm./L. in agar cup-plate tests or by 0.01 Gm./L. when the compound was incorporated in the nutrient substratum. The compound is strongly antagonistic also to *Microsporum canis*, *M. audouini*, and *Botrytis allii*. Concentrations of 25 mg. or more of K₈/L. checked fermentation by yeast (*Saccharomyces cerevisiae*) in a 10 per cent glucose solution.

The results are interpreted in terms of altered redox potentials.

The possible use of vitamin K₈ in clinical treatment of dermatomycoses and as a preservative in the food and beverage industries is suggested.

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INACTIVATION OF ENZYME-SUBSTRATE FILMS BY SMALL DOSES OF X-RAYS

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The analysis of the biological effects of radiations in terms of their effects on simpler biochemical systems is rendered difficult by the fact that most biochemical systems seem so insensitive to radiations. In particular, chromosomes are often affected, structurally and genetically, by doses smaller by orders of magnitude than those which affect enzyme solutions, virus preparations, etc., measurably. This apparent paradox contains certain clues for the further analysis of biological effects. It focuses attention on the chromosomes as the sensitive agents in the cell. This apparent sensitivity is based in part on method. Chromosomal effects are often expressed as visible changes, so that a single effect may be detected. The genetic units in the chromosome are unique, so that the consequences of a single event at the molecular level can be far-reaching and persistent. This contrasts with the statistical nature of most chemical measurements.

But the question of the radiation sensitivity of chromosomes involves factors not represented in an enzyme or virus solution, most clearly evident where a single radiation event produces a break in a microscopic structure. The chromosome is a continuous "solid" body, varying in both structure and sensitivity through its own cycle and according to experimental conditions. In attempting to visualize the effects of radiation, the structural factor has to be introduced. Therefore it would be desirable to set up a biochemical system in which the factor of intermolecular structure was present and in which the variables encountered by the chromosomes could be tested. If such a system showed a high radiation sensitivity, this would in itself suggest a relation between structure and sensitivity.

We have, in accordance with these requirements, investigated the radiation sensitivity of surface films containing a proteolytic enzyme and its substrate. Mazia, Hayashi and Yudowitch¹ have described the structure and enzymatic activity of such a system involving pepsin and albumin, and have considered possible parallels with chromosome structure and activity. Preliminary experiments showed that this system was indeed sensitive to radiations. X-ray doses of the 50-150 r order produced extensive inactivation.

The sensitivity seemed to depend on the structural configuration, for the same doses applied to the solutions from which the films were prepared had no detectable effect. The sensitivity might depend on alterations of the molecules on spreading, on the participation of sensitive intermolecular bonds, not present in the solution, in the reaction, or merely on the geometry of the exposure of the films to the irradiated medium. All of these possibilities may also be implicated in the sensitivity of chromosomes, and all of them would be absent in experiments on solutions. The present series of investigations is an exploration of these structural factors in radiation sensitivity. The possible analogies between the actual structure of the films and of chromosomes are not essential to such an exploration.

Methods.—Each experiment consists of the following steps. (1) A film containing both enzyme and substrate is prepared by spreading at a pH at which the activity of the enzyme is negligible. (2) The film is irradiated. (3) The film is compressed into a fiber which is removed and washed. (4) The enzymatic activity in the fiber is estimated by bringing the fiber to the pH optimum of the enzyme and timing its self-digestion as observed microscopically.

Solutions.—The starting solutions consisted of mixtures of crystalline egg albumin, prepared by the method of LaRosa,² and crystalline pepsin (Lehn and Fink Co.) in water. The albumin was dialyzed in the cold and frozen dried. As this treatment produced pepsin preparations of varying activity, we used the pepsin-MgSO₄ mixture without further purification, estimating its protein content by the method of Robinson and Hogden.³ The total protein concentration used was 0.2 per cent. A small amount of isopropyl alcohol was used to improve spreading.

Preparation of Films.—The films were spread over 10-fold diluted MacIlvaine buffer, the final pH being 4.0, in a Cenco Hydrophil tray. Pyrex-redistilled water was used. The protein solution was measured onto the previously swept surface with a 0.1 ml. pipette. In most of the experiments, 0.05 ml. of the solution, a slight excess over what would be required to cover the surface under ideal conditions, was used. This meant that there was a small amount of surplus protein, about 0.03 mg. of albumin and about 0.001 mg. of pepsin, dissolved in the liter or so of buffer under the film. The buffer was changed for each experiment, so that there would be no accumulation of dissolved protein. The possibility of misinterpretation of the results because of the presence of dissolved enzyme will be considered later.

Irradiation.—The film trough was placed under the x-ray tube so that the target was directly over the center of the film. The standard area irradiated was 18 × 32 cm. The dose rate was controlled by varying the target-to-film distance, but, to insure covering the film, this was never less than 20 cm.

The Coolidge tube was operated at 150 kv. and 4 amperes with a 1-mm.

Al filter. Dose was not measured for each experiment, but was calculated from calibrations made with a Victoreen dosimeter set at various points in the film tray, the tray being filled with paraffin. By this means, allowance could be made for back-radiation, and for variations inherent in the geometry of the system. Doses given in the data below are those immediately below the target, and therefore represent the maximum received by the films.

Test of Activity.—The activity of pepsin-albumin films may be detected and measured microscopically. The film is compressed into a fiber by bringing the barriers together, and the fiber is removed and washed as free as possible of solutes from the trough with distilled water. The washed fiber is then placed in HCl at pH 1.5 and observed microscopically. Fibers containing active pepsin are observed to digest themselves and disappear rapidly. Control fibers containing albumin and pepsin in a 40 to 1 ratio digest in 30 to 50 seconds at pH 1.5 and 25°C. Mazia and Hayashi⁴ have tested this microscopic method by comparison with measurements on the rate of liberation, under similar conditions, of trichloroacetic-soluble split products, following the methods of Anson.⁵ The results have invariably been parallel; the slower the disappearance of the fibers, the lower is the rate of release of split products. While the time required for visible digestion may not be a precise measure of the *rate* of digestion, we have never encountered a case where visible digestion was not accompanied by release of split products or where a fiber that did not visibly digest gave a full yield of split products. We consider, therefore, that a slowing down or complete inhibition of visible digestion is a measure of inactivation, and this was the measure used in testing the effects of irradiation. For the present, our chemical method is not suitable for the type of experiment described in this paper, since it requires about 1 mg. of dried fiber.

Results.—Rate of Digestion as a function of x-ray Dose.—The effects of radiation on digestion-time are shown in figure 1. The two curves represent films prepared from solutions containing pepsin-albumin ratios of 20:1 and 40:1. Each point represents a completely independent experiment. We shall not at this time consider the source of variability in the observations, except to mention that later work indicates that sensitivity is very dependent on surface pressure which was not controlled in the experiments shown in figure 1. The trend of the results is clear enough. Effects of doses of the order of 50 r may be detected and doses over 150 r produce the maximum effect that can be observed: complete failure of the compressed film to digest. The results suggest very strongly a "threshold" effect. In terms of delay in digestion time, doses below 50 r are not very effective compared with the same increments of dose above 50 r.

Measurement of Effect.—While the results given do show that we are dealing with a highly radiosensitive enzyme system, the bare data do not

permit a quantitative evaluation of the effect. For this purpose it would be desirable to express the results as the proportion or number of active units

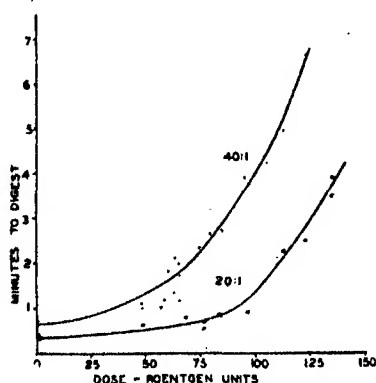


FIGURE 1

Relation between digestion rate and x-ray dose. Each point represents an independent experiment. Ratios are albumin-pepsin ratios in solutions from which films were prepared.

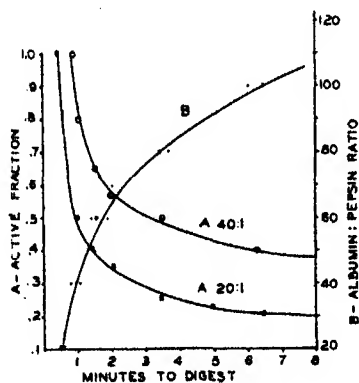


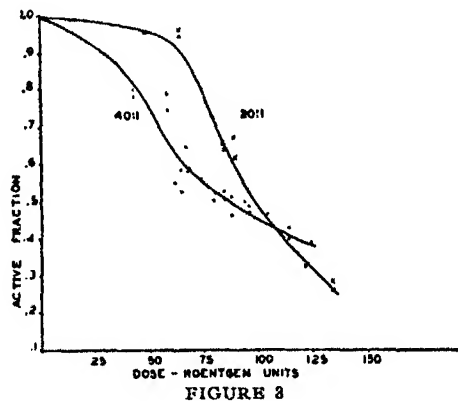
FIGURE 2

Relation between enzyme content of films and digestion rate. Curve *B* represents digestion rates for various albumin pepsin ratios (Scale *B*). Ratios are those in solutions from which films were prepared. Curves *A* (read against scale *A*) are calculated from Curve *B*. Enzyme content of 20:1 and 40:1 films are taken as unity, digestion rate is plotted against fractions of initial enzyme content.

affected. This is possible if a relation can be found between the number of active units and the digestion rate. Such a "calibration" is obviously possible, for all that is necessary is to determine the digestion rate of films pre-

pared from solutions containing various substrate enzyme ratios. The results of such a calibration are given in figure 2.

The use of this calibration depends on certain assumptions. First, it has to be assumed that there is a definite relation between the enzyme and substrate concentrations in the solutions and in the film. It is assumed, in the plot, that the ratios in the film approximate those in the solutions. Second, it must be assumed that any decrease in the digestion rate after irradiation results from the inactivation of a certain proportion of enzyme molecules rather than other conceivable causes. This assumption is difficult to test. It is, however, present in every enzyme experiment where the amount of active enzyme is deduced by comparison with a concentration-activity curve. The only alternative would be the impracticable one of isolating the active fraction.



Relation between x-ray dose and surviving active fraction of enzyme in film. Data from figure 1 are replotted using calibration data of figure 2.

In figure 2, Curve B represents the actual relation between the substrate enzyme ratio and the rate of digestion, as determined by separate experiments on various mixtures. The other curves are based on the same data and are introduced merely for convenience. They represent the relation between the active fraction of the enzyme and digestion rate where certain substrate-enzyme ratios are taken as unity.

These curves may then be used to express the radiation data of figure 1 in terms of the surviving fraction of the initial activity. Obviously, they can also be used to calculate the number of units inactivated, using the area of the film and the molecular weights of the proteins.

Figure 3 shows the radiation data plotted as dose against active fraction. If the calibration of the method is valid, this plot establishes the high sensitivity of the system in an absolute sense, for it is seen that a dose of 100 r

destroys more than half of the activity present. Certain other points are suggested by the plot.

1. The "threshold" effect suggested by figure 1 remains. The point of deflection is difficult to determine as might be expected where individual films vary in sensitivity.

2. There is an unexplained difference between the two films of different composition. The threshold effect is less pronounced in the film with a 40:1 substrate enzyme ratio than in the 20:1 film. Moreover the whole dose-effect relationship is different for the two films. If we were dealing with a "hit" relationship, we could expect the curves to coincide, since the fraction affected would not be a function of the size of the population. If we were dealing with an indirect action as described by Dale, it would be expected

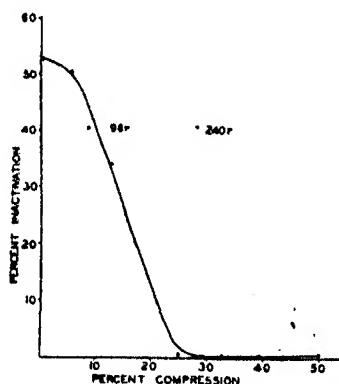


FIGURE 4

Relation between surface compression and radiation sensitivity. Compression given in units of area, not pressure. Inactivation calculated from calibration in figure 2. Curve represents inactivation by 96 r. The single point gives inactivation by 240 r at 28 per cent compression.

that the total number of units affected by a given dose would be independent of the number present. This is not the case. It is obvious that the composition affects the sensitivity in a way that is not simple.

Effect of Compression on Sensitivity.—These films are more sensitive to radiation than would be predicted from their composition alone, and the structural factors in their sensitivity have to be taken into account. One possibility is that the spreading of the enzyme molecule (however incomplete) may increase its sensitivity. Another is that the structural continuity may be a factor. These variables may be tested by determining the effect of surface compression on radiation sensitivity. In these experiments, the films were spread as already described, compressed to a given area, irradiated, then fully compressed and tested for digestion rate. The results are

given in figure 4. There is evidently a sharp, discontinuous effect. Sensitivity begins to fall off at about 10 per cent compression, and at about 28 per cent compression is lowered to the point where the effects of a dose of 96 r are not detectable. Sensitivity is only lowered, not destroyed. At 28 per cent compression, 240 r produce about the same effect as 96 r at 13 per cent compression. If area of exposure to irradiated medium determined the sensitivity, one would expect some simple proportionality between area and radiation effect instead of the discontinuous relationship observed. It seems clear that, regardless of the mechanism of the radiation effect, the effect is on the film itself rather than on dissolved enzyme and that the sensitivity is determined by the physical configuration of the film. Obviously, the "compression" shown in the data is only a very rough index of changes in surface pressure, and it is necessary to investigate the relationship between actual pressure and sensitivity. Such an investigation is now under way.

Calculations.—Although the experiments bearing on the mechanism of x-ray action on the enzyme substrate films will be reported in a second paper, the nature of the problem can be indicated by some preliminary calculations. In making these, we have relied on the tables computed by Lea.⁶ First, the number of units affected may be estimated from figure 3. The film area used in our experiments was 576 cm.² Film pressure determinations show this to contain about 0.07 mg. of protein, or a total of 10^{16} molecules of molecular weight 34,000. A 20:1 albumin pepsin film would contain about 5×10^{13} pepsin molecules and a 40:1 film half this number. In orders of magnitude, 100 r, according to figure 3, would produce inactivation equivalent to the removal of 10^{13} pepsin molecules.

The expected number of ionizations in the film itself may be estimated on the basis of the mass of protein, neglecting geometric considerations which would make the true figure lower than the estimate. For the film containing 0.07 mg. of protein, 100 r of 0.15 Å x-rays would yield about 10^{10} ionizations, a figure lower by orders of magnitude than the estimated number of pepsin molecules inactivated.

If the effect is through ionization products in the medium, the calculation has to be made on the basis of unstable products of water decomposition. Experiments in which films were spread over solution that had previously received large doses of radiation showed no differences from controls, and therefore the formation of adequate concentrations of stable products must be excluded. Using Lea's data again, it is calculated that the number of radicals produced by 100 r is of the order of 10^{14} per cc. One may calculate the time that would be required for 10^{13} radicals to reach the film if they were stable. The equation for diffusion across a plane from a semi-infinite column into a column of zero concentration is given by Jacobs⁷ (equation 104). We use as diffusion constant the value of 2×10^{-6} cm.² sec.⁻¹, the

constant for D_2O in H_2O . The calculation shows that it requires 10^{-4} – 10^{-5} seconds for 10^{12} radicals to reach the surface. If, then, radicals produced by the decomposition of water were the effective agents, the simple calculation would require their half-life to be of this order. If only radicals finding a pepsin molecule were effective, the time would be 20–40 times greater. Lea estimates 10^{-7} seconds as their half-life in water, and less in the presence of solutes such as citric acid which was present in 0.01 *M* concentration in our experiments.

On the face of these considerations, our results would require the assumption that the effects of a single radiation event, whether in direct action through ionization or indirect action through ionization products, are spread over a considerable number of pepsin molecules.⁸ These calculations alone, however, cannot be considered to be a demonstration of the spread of the effect. Lea's estimate of the half-life of radicals produced by irradiation of water may be too low. Moreover, it may not be valid to base the number of inactivations on numbers of pepsin molecules. The film may in fact be a complex polymer, where the scope of a unit inactivation would be difficult to define.

Discussion.—We have restricted ourselves to the description of the radiation sensitivity of the film enzyme-substrate system, and will present data bearing on the mechanism of the effect in a subsequent publication. The basic fact is that the pepsin-albumin films described are unexpectedly sensitive to x-rays, doses of less than 200 r causing the maximum measurable inactivation and doses of the order of 50 r producing readily detectable inactivation. In absolute terms, this sensitivity exceeds that of biological systems, where we do not obtain, with such doses, such large percentages of all possible effects.

The dose-effect curves, with the indicated "threshold" effect, suggest nothing so much as "multiple hit" curves, but their analysis must await the decision as to the type of mechanism causing the effect.

The interest of these observations rests not so much on the sensitivity itself, which might conceivably be duplicated in dilute pure solutions, as on the fact that the sensitivity is obtained in a situation conceptually comparable to that in chromosomes. By organizing the enzyme system into a solid film we are liberated from the restrictions of a dilute solution. The films are as "concentrated" as can be imagined and may contain any number of components. The only possible "protective" action that need be considered is that of the solutes in the adjacent liquid phase. Without pressing detailed analogies between films and cellular structures, it is obvious that with the films certain variables arising in cytological problems may be investigated at a biochemical level. Examples are the question of the spreading effect of a single radiation event within a structure, the range of ionization products, and, particularly, the relation between physical structure and

radiation sensitivity. The aim of further work along these lines is to find ways of picturing biological effects of radiation and premises to be used in their interpretation.

Summary.—(1) Pepsin-albumin films may be inactivated by small doses of x-rays. Doses of about 100 r produce about 50 per cent inactivation.

(2) The sensitivity to radiation depends on the physical configuration of the molecules. It may be varied by surface compression.

(3) Calculations suggest that the effects of a single radiation event (ionization or radical production) may be spread to include a large number of enzyme molecules.

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⁵ Anson, M. L., *J. Gen. Physiol.*, 22, 79 (1938-1939).

⁶ Lea, D. E., *Action of Radiations on Living Cells*, Cambridge, 1947.

⁷ Jacobs, M. H., *Ergeb. d. Biol.*, 12, 1 (1935).

⁸ J. Weiss (*Brit. J. Radiol., Suppl. No. 1*, 56 (1947)) has recently proposed that a radical produced by the ionization of solvent may initiate a chain reaction extending as far as 10^{-3} cm.

NOTES ON INTEGRATION, I

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The theory of integration, because of its central rôle in mathematical analysis and geometry, continues to afford opportunities for serious investigation. The need for extending and rounding out the classical studies of Riemann, Stieltjes and Lebesgue has stimulated considerable interest not only in new aspects of the theory but also in the simplification and perfection of the old. The present series of communications is intended to outline a treatment which, while exploiting fully the possibilities for simplification, will attain a high degree of generality. Among the many contributions to the mathematical literature which have provided material for our handling of the subject we wish to cite above all an important paper of Daniell.¹ In spite of the fact that the basic ideas in the

present discussion are the common property of mathematicians, some of our results appear to be novel. If they are, it is because we have chosen to introduce and exploit an adaptation of the concept of an "upper integral," making this the technical foundation of the whole theory.³

With Daniell, we assume an initially given *elementary integral* $E(f)$ which is defined for a non-void class \mathfrak{E} of real functions f , called the *elementary functions*, with a fixed abstract set X as domain. Specifically, our basic postulates are the following slight modifications of those given by Daniell:

(1) \mathfrak{E} is a non-void class of real functions f on an arbitrary non-void domain X , such that αf , $f + g$, and $|f|$ are in \mathfrak{E} whenever α is a real number and f and g are both in \mathfrak{E} ; and E is a real-valued function (or operation) defined over \mathfrak{E} , such that

$$E(\alpha f) = \alpha E(f), \quad E(f + g) = E(f) + E(g), \quad E(|f|) \geq 0;$$

(2) if f and f_n are in \mathfrak{E} and $|f| \leq \sum_{n=1}^{\infty} |f_n|$, then

$$E(|f|) \leq \sum_{n=1}^{\infty} E(|f_n|).$$

At a certain stage in the development, we shall introduce the further requirement that

(3) if f is in \mathfrak{E} , then $\min(1, f) = \frac{1}{2}(1 + f - |1 - f|)$ is in \mathfrak{E} .

Technically (1) means that \mathfrak{E} is a vector lattice under its natural ordering and E is a positive linear functional on \mathfrak{E} . The rôles played by (2) and (3) will be brought out below.

In many important instances of the general theory, \mathfrak{E} consists of the continuous real functions with compact nucleus³ on a locally compact space X . Under these conditions (2) and (3) both follow from (1): (3) is evident, while (2) is established by constructing a non-negative elementary function g strictly positive on the nucleus of f and demonstrating the existence of an integer $m = m(\epsilon)$, $\epsilon > 0$, such that $|f| - \epsilon g \leq \sum_{n=1}^m |f_n|$.

By way of illustration we cite the Riemann integral on a bounded closed domain in n -space or on an n -dimensional manifold, and the Haar integral on a locally compact topological group.⁴ Other important instances of the general theory are constructed from distributions of simple type given in advance on X : in X a class of subsets is distinguished and each distinguished set Y is assumed to bear a non-negative weight or measure, $\mu(Y)$; the real linear lattice combinations of the characteristic functions of distinguished sets are taken as the elementary functions; and the elementary integral is then determined in harmony with the requirement that $E(f) = \mu(Y)$ for the characteristic function f of any distinguished set Y . The detailed construction is not difficult once the appropriate tech-

niques have been provided for relating the data to the requirements of (1) and (2). We mention in particular that the given measure must be additive: if Y , Z , and $Y \cup Z$ are distinguished sets, and Y and Z are disjoint, then $\mu(Y \cup Z) = \mu(Y) + \mu(Z)$. The theory of the Lebesgue-Stieltjes integral illustrates the general concepts which have just been described.

We shall consider the class \mathfrak{G} of all extended-real functions defined on the domain X . The admission of $+\infty$ and $-\infty$ as functional values renders a little awkward the definition of such algebraic combinations of functions as $0 \cdot f$, $f + g$ and $f - g$. In the present context it is appropriate to let $0 \cdot f$, $f + g$, $f - g$ designate (ambiguously!) any function in \mathfrak{G} which assumes the respective values $0 \cdot f(x)$, $f(x) + g(x)$, $f(x) - g(x)$ at every x where the latter quantities are defined. As we shall see, this ambiguity raises no serious obstacles in the sequel.

Over \mathfrak{G} we define an extended-real function N by putting

$$(4) \quad N(f) = \inf \{ \lambda; \lambda = \sum_{n=1}^{\infty} E(|f_n|), |f| \leq \sum_{n=1}^{\infty} |f_n|, f_n \in \mathfrak{G} \}.$$

For non-negative functions, $N(f)$ has the properties of an upper integral; and for certain non-negative functions it coincides with the integral which is presently to be defined. Thus we already have in our possession the mathematical object upon which our interest is concentrated. The formal definition given in (4) is capable of an informal presentation which brings out clearly and rather simply its intuitive justification. Confining our attention to non-negative functions, we may regard (4) as the condensed description of a measuring process. We may think of \mathfrak{G} as providing a stock of measuring rods, the non-negative elementary functions, by means of which the non-negative functions in \mathfrak{G} are to be gauged. Each measuring rod has a magnitude given by its elementary integral. The basic measuring process consists in choosing from stock such an infinite sequence of measuring rods $f_n = |f_n| \geq 0$ that by addition they combine to surpass $f = |f| \geq 0$ in the sense indicated by the inequality

$$f \leq \sum_{n=1}^{\infty} f_n. \quad \text{The real number } \lambda = \sum_{n=1}^{\infty} E(f_n) \text{ obtained by adding together}$$

the magnitudes of the particular measuring rods thus employed is then accepted as an estimate, generally in excess, of the magnitude of f . Repetitions of this basic process furnish successively better estimates, convergent to the quantity $N(f)$, when suitable precautions are taken. It is, of course, conceivable that the basic process will fail to produce any real numbers as estimates of the magnitude of a particular function f —either because the inequality $f \leq \sum_{n=1}^{\infty} f_n$ cannot be realized, or because it

implies the divergence of the series $\sum_{n=1}^{\infty} E(f_n)$. Under these circumstances the formal definition requires that we take $N(f) = +\infty$ in accordance with a well-known convention. The principal properties of the function N , all easily deduced from (4), can be listed as follows:

$$(5) \quad 0 \leq N(f) \leq +\infty;$$

$$(6) \quad N(\alpha f) = |\alpha| N(f) \text{ unless } \alpha = 0 \text{ and } N(f) = +\infty;$$

$$(7) \quad |f| \leq \sum_{n=1}^{\infty} |f_n| \text{ implies } N(f) \leq \sum_{n=1}^{\infty} N(f_n);$$

$$(8) \quad N(|f|) = N(f).$$

By specializing (7) we see that $N(f + g) \leq N(f) + N(g)$, and that $N(f) \leq N(g)$ whenever $|f| \leq |g|$. The ambiguity of the expressions αf , $f + g$ does not affect the truth of these relations; nor does (2) enter into their proofs. It is only in deriving the property

$$(9) \quad \text{when } f \text{ is elementary, } N(f) = E(|f|)$$

that (2) finds any direct application in our theory. Clearly (2) and (9) are equivalent assertions, so that whenever we use (9) in the sequel we also use (2) in an essential though implicit manner.

Henceforth we shall be concerned primarily with that part, \mathfrak{F} , of \mathfrak{G} which is characterized by the inequality $N(f) < +\infty$. The expression $N(f - g)$ has in \mathfrak{F} the properties of a pseudo-metric. Hence, if we identify functions f and g for which $N(f - g) = 0$, we can treat \mathfrak{F} as a real normed vector-lattice with N as its norm-function. The detailed discussion involves attention to those functions f , called *null functions*, for which $N(f) = 0$. In this connection it is convenient to introduce also the following definitions: a subset of X is called a *null set* if its characteristic function is a null function; the phrase "*almost everywhere*" signifies "with the exception of the points of a certain null set." By the use of (7) it is easy to show that a function is a null function if and only if it vanishes almost everywhere; that any set covered by a countable family of null sets is a null set; and that every function in \mathfrak{F} is finite almost everywhere. These and similar properties show that the null sets play here a rôle analogous to that played by the sets of measure zero in the Lebesgue theory. The identification of functions in \mathfrak{F} is now seen to remove all ambiguity in the meaning of the expressions αf , $f + g$, $f - g$ since every function in \mathfrak{F} is finite almost everywhere. Once these more or less routine matters are disposed of, we can establish the most important single result⁶ concerning \mathfrak{F} , namely:

(10) the normed vector space \mathfrak{F} is complete (and hence a Banach space).

The proof will be sketched. Let $\{f_n\}$ be a Cauchy sequence in \mathfrak{F} . Without loss of generality we may suppose that f_n is everywhere finite (otherwise we could modify f_n on a null set so that the resulting function would be everywhere finite) and that $N(f_{n+1} - f_n) \leq 2^{-n}$ (otherwise we could choose a subsequence which converges with the desired rapidity).

The series $|f_1| + \sum_{n=1}^{\infty} |f_{n+1} - f_n|$ has the sum g in \mathfrak{G} . Since $N(g) \leq N(f_1) + \sum_{n=1}^{\infty} N(f_{n+1} - f_n) = N(f_1) + \sum_{n=1}^{\infty} 2^{-n} \leq N(f_1) + 1 < +\infty$ by (7), g is actually in \mathfrak{F} . Hence the series above converges almost everywhere, and so also do the series $f_1 + \sum_{n=1}^{\infty} (f_{n+1} - f_n)$ and the sequence $\{f_n\}$. Let f be any function in \mathfrak{G} which is equal to the sum of the latter series or, equivalently, to $\lim_{n \rightarrow \infty} f_n$ wherever these quantities are defined: we may, for example, take $f = \limsup_{n \rightarrow \infty} f_n$ or $f = \liminf_{n \rightarrow \infty} f_n$. We then have $|f| \leq g$, $N(f) \leq N(g) < +\infty$, $f \in \mathfrak{F}$. Moreover $|f - f_k| \leq \sum_{n=k}^{\infty} |f_{n+1} - f_n|$ and $N(f - f_k) \leq \sum_{n=k}^{\infty} N(f_{n+1} - f_n) \leq 2^{-k+1}$, so that the Cauchy sequence $\{f_n\}$ has f as its limit in \mathfrak{F} . This completes the proof.

On \mathfrak{F} we now define the function F by putting $F(f) = N(f^+) - N(f^-)$ where $f^+ = \frac{1}{2}(|f| + f) \geq 0$, $f^- = \frac{1}{2}(|f| - f) \geq 0$. Since $|f^+ - g^+| \leq |f - g|$ and $|f^- - g^-| \leq |f - g|$, this function is continuous in accordance with the inequalities $|F(f) - F(g)| \leq |N(f^+) - N(g^+)| + |N(f^-) - N(g^-)| \leq N(f^+ - g^+) + N(f^- - g^-) \leq 2N(f - g)$. On $\mathfrak{G} \subset \mathfrak{F}$ we have $F(f) = E(f^+) - E(f^-) = E(f)$ by (9). Let \mathfrak{L} be the closure of \mathfrak{G} in \mathfrak{F} , and let L be the contraction of F to \mathfrak{L} . A function in \mathfrak{L} is said to be *integrable*, and its *general integral* is taken to be $L(f) = N(f^+) - N(f^-)$. The integrable functions are the functions which can be approximated by elementary functions in the sense of the norm for \mathfrak{F} . On the other hand, the results of the preceding paragraph show that every integrable function f is equal almost everywhere to $\limsup_{n \rightarrow \infty} f_n$, where $\{f_n\}$ is a suitably chosen sequence of elementary functions. The following assertions concerning the general integral can now be justified:

- (11) \mathfrak{L} and L enjoy the properties assumed for \mathfrak{G} and E , respectively, in (1), \mathfrak{L} being a complete vector subspace of \mathfrak{F} with norm $N(f) = L(|f|)$;
- (12) the sum of a positive-term series of integrable functions is integrable if and only if the corresponding series of integrals converges (necessarily to the integral of the sum-function).

It is clear that (11) follows from (1) by simple continuity arguments. We observe that (12) may be regarded as a sharpened version of (2) formulated in terms of \mathfrak{L} and L ; and also that (12) is a generalized form of the

theorem of B. Levi in the Lebesgue theory. A proof of (12) will now be sketched. Let $f = \sum_{n=1}^{\infty} f_n$ where $f_n \geq 0$ and $f_n \in \mathfrak{L}$. If $f \in \mathfrak{F}$, then $\sum_{n=1}^m L(f_n) = L(\sum_{n=1}^m f_n) = N(\sum_{n=1}^m f_n) \leq N(f) < +\infty$ so that $\sum_{n=1}^{\infty} L(f_n)$ is a convergent positive-term series. On the other hand, if $\sigma = \sum_{n=1}^{\infty} L(f_n) < +\infty$, we have $N(f) \leq \sum_{n=1}^{\infty} N(f_n) = \sum_{n=1}^{\infty} L(f_n) < +\infty$ so that $f \in \mathfrak{F}$. Moreover $N(f - \sum_{n=1}^m f_n) \leq \sum_{n=m+1}^{\infty} N(f_n) = \sum_{n=m+1}^{\infty} L(f_n)$ so that $\sum_{n=1}^m f_n$ converges in \mathfrak{L} to f and $L(\sum_{n=1}^m f_n)$ converges to $L(f)$, with the result that $L(f) = \sigma$. From (12) it is now possible to deduce other convergence theorems which correspond in our general theory to such standard results as the Lebesgue dominated-convergence theorem and the Fatou theorem in the Lebesgue theory. As the classical arguments apply without change to the present situation we do not need to go into detail.

Let Φ_m be the class of all positively homogeneous continuous real functions of m real variables $\lambda_1, \dots, \lambda_m$. To Φ_m belong the functions $\lambda_1, \dots, \lambda_m$ and all the linear lattice combinations which can be formed from them. On the other hand, it is known that on the compact set where $|\lambda_1| + \dots + |\lambda_m| = 1$ any continuous function can be uniformly approximated by such combinations.⁶ Hence if $\varphi \in \Phi_m$ we can find such a combination ψ_k that $|\varphi - \psi_k| \leq 1/k$ on this set. It follows that $|\varphi - \psi_k| \leq 1/k (|\lambda_1| + \dots + |\lambda_m|)$. Since $\psi_k(f_1, \dots, f_m)$ is integrable when f_1, \dots, f_m are, as we noted in (11), and since

$$N(\varphi(f_1, \dots, f_m) - \psi_k(f_1, \dots, f_m)) \leq (N(f_1) + \dots + N(f_m))/k \rightarrow 0$$

when $k \rightarrow \infty$, we conclude that $\varphi(f_1, \dots, f_m)$ is integrable. The dominated-convergence theorem enables us to extend this result to the positively homogeneous Baire functions:

(13) if φ is a positively homogeneous real function of m real variables $\lambda_1, \dots, \lambda_m$ whose contraction to the set $|\lambda_1| + \dots + |\lambda_m| = 1$ is a bounded Baire function, and if f_1, \dots, f_m are integrable, then $\varphi(f_1, \dots, f_m)$ is integrable.

In order to rid ourselves of the restriction to homogeneous functions, we now assume that (3) holds.⁷ We find that (13) can be replaced by:

(14) if φ is a finite, not necessarily bounded Baire function of m real variables $\lambda_1, \dots, \lambda_m$ such that $\varphi(0, \dots, 0) = 0$, and if f_1, \dots, f_m, g are integrable functions such that $|\varphi(f_1, \dots, f_m)| \leq |g|$, then $\varphi(f_1, \dots, f_m)$ is integrable.

Indeed, if the constant function everywhere equal to 1 is in \mathfrak{E} or in \mathfrak{L} , we can also eliminate the condition $\varphi(0, \dots, 0) = 0$. By further applica-

tion of the dominated-convergence theorem we can extend (13) and (14) to functions φ of infinitely many variables.

The principal processes for manipulating the general integral have now been justified. In our second note we shall review some of their applications and consequences.

¹ Daniell, P. J., "A General Form of Integral," *Ann. Math.*, **19**, 279-294 (1917-1918).*

² Some remarks of H. Blumberg, *Am. Math. Monthly*, **53**, 189 (1946), on Lebesgue measure shed much light on the subject for me and started a train of thought culminating in the introduction of an "upper integral" here.

³ The nucleus of a function is the closure of the set of points where it assumes non-zero values.

⁴ Weil, A., *L'Intégration dans les Groupes Topologiques et ses Applications*, Paris, 1938, 34-38.

⁵ This result is believed to be new.

⁶ Stone, M. H., "The Generalized Weierstrass Approximation Theorem," *Math. Mag.* **21**, 167-183 (1948); see particularly §2, where Corollary 2 to Theorem 3 gives the relevant information.

⁷ Other conditions leading to (14) have been investigated by Mr. H. Rubin, who, as a member of one of my classes, made many useful comments on the subject-matter of this whole paragraph.

ON THE DIFFERENTIAL EQUATIONS OF SLIP FLOW

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In a compressible medium, let the stress tensor be T_j^i , the specific internal energy ϵ , the specific entropy s , the specific volume σ . Then the *mean pressure* p_m and the *pressure* p are given by the definitions

$$p_m \equiv -\frac{1}{3} T_{ii}^i \quad p \equiv -\left(\frac{\partial \epsilon}{\partial \sigma}\right)_s \quad (1)$$

Let V_i be the velocity vector; then the *deformation* and *rotation* tensors d_{ij} and ω_{ij} , respectively, are given by the definitions

$$d_{ij} \equiv \frac{1}{2} (V_{i,j} + V_{j,i}), \quad \omega_{ij} \equiv \frac{1}{2} (V_{i,j} - V_{j,i}). \quad (2)$$

If a secondary stress tensor W_j^i be given by the definition

$$W_j^i \equiv p \delta_j^i + T_j^i, \quad (3)$$

then the *dissipation function* Φ , given by the definition

$$\Phi = W_j^i d_{ij}^j, \quad (4)$$

may be shown to be the rate of conversion of mechanical energy into thermal energy per unit volume.

A *compressible fluid* may be defined as a continuous medium obeying the following postulates:

I. There exist material constants μ_0 , θ_0 , and \Re such that

$$\begin{aligned}\dim \mu_0 &= \frac{\text{mass}}{(\text{length}) (\text{time})}, \\ \dim \theta_0 &= \text{temperature}; \\ \dim \Re &= \frac{(\text{length})^2}{(\text{time})^2 (\text{temperature})}.\end{aligned}$$

II. $\Phi = \Phi(\mu_0, \theta_0, \Re; p_m; p, \theta; p_{,i}, \theta_{,i}, f_{i,j}, p_{,ij}, \theta_{,ij}; \dots; f_{i_1 i_2} \dots i_m, p_{,i_1 i_2} \dots i_m, \theta_{,i_1 i_2} \dots i_m; d_{ij}, \omega_{ij}; c_{(1)} M_{(1)}, c_{(2)} M_{(2)}, \dots, c_{(k)} M_{(k)})$

III. $W_j^i = 0$ if $V_{i,j} = 0, p_{,i} = 0, \theta_{,i} = 0, f_{i,j} = 0$.

In the above definition, θ is the temperature, f_i is the extraneous force per unit volume, $c_{(j)}$ is the molal concentration and $M_{(j)}$ is the molecular weight of the j th of the k phases of the various substances present, and m is an arbitrary positive integer.

If it be supposed that the function Φ is an analytic function of all its tensorial arguments (though not necessarily also of its scalar arguments), then it is possible to show that the definition just given leads to an expression for the stress tensor W_j^i of the general form

$$W_j^i = \sum_{r=1}^{\infty} \mu_r W_j^i, \quad (5)$$

where the quantity μ_r

$$\mu_r = \mu_0 f \left(\frac{p}{p_m}, \frac{\theta}{\theta_0}, c_{(1)} M_{(1)}, c_{(2)} M_{(2)}, \dots, c_{(k)} M_{(k)} \right), \quad (6)$$

the function f being a dimensionless function of its arguments, may be identified with the ordinary coefficient of viscosity. In the special case of an *isotropic* fluid it may then be shown that

$${}^{(1)} W_j^i = \frac{\lambda}{\mu} d_k^k \delta_j^i + 2d_j^i, \quad (7)$$

$$\begin{aligned}{}^{(2)} W_j^i &= \frac{\Re \theta}{p_m^3} [D_{(1)} p^k p_{,k} \delta_j^i + 2C_{(1)} p^i p_{,j}] + \frac{\Re}{p_m^2} [D_{(3)} p^k \theta_{,k} \delta_j^i + \\ &\quad C_{(2)} (p^i \theta_{,j} + \theta^i p_{,j})] + \frac{\Re}{p_m \theta} [D_{(3)} \theta^k \theta_{,k} \delta_j^i + 2C_{(3)} \theta^i \theta_{,j}] + \\ &\quad \frac{\Re \theta}{p_m^2} [D_{(4)} p^k \delta_j^i + 2C_{(4)} p_j^i + D_{(5)} f_k^k \delta_j^i + C_{(5)} (f_j^i + f_j^i)] +\end{aligned}$$

$$\begin{aligned}
& \frac{\Re}{\rho_m} [D_{(6)} \theta_k^k \delta_j^i + 2C_{(6)} \theta_j^i] + \frac{1}{\rho_m} [F_{(1)} (d_k^k)^2 \delta_j^i + F_{(2)} d_k^i d_l^k \delta_j^i + F_{(3)} \omega_k^i \omega_l^k \delta_j^i + \\
& \quad 2E_{(1)} d_k^k \delta_j^i + 2E_{(2)} d_k^i d_j^k + 2E_{(3)} \omega_k^i \omega_j^k + E_{(4)} (d^{ik} \omega_{kj} + d_{jk} \omega^{ki})], \quad (8) \\
& {}_{(3)}W_j^i = \frac{\Re \theta}{\rho_m^4} [G_{(1)} d_k^k p_{,i} p_{,j} \delta_j^i + G_{(2)} d^{k1} p_{,i} p_{,k} \delta_j^i + G_{(3)} (d^{ik} p_{,k} p_{,j} + \\
& \quad d_{jk} p^{,k} p^{,i}) + G_{(4)} d_j^i p^{,k} p_{,k} + H_{(1)} (\omega^{ik} p_{,k} p_{,j} + \omega_{jk} p^{,k} p^{,i})] + \\
& \frac{\Re}{\rho_m^3} [G_{(5)} d_k^k p_{,i} \theta_{,j} \delta_j^i + G_{(6)} d^{k1} p_{,k} \theta_{,i} \delta_j^i + G_{(7)} (d^{ik} p_{,k} \theta_{,j} + d_{jk} p^{,k} \theta^{,i}) + \\
& \quad G_{(8)} (d^{ik} \theta_{,k} p_{,j} + d_{jk} \theta^{,k} p^{,j}) + G_{(9)} d_j^i p^{,k} \theta_{,k} + H_{(2)} \omega^{k1} p_{,k} \theta_{,j} \delta_j^i + \\
& \quad H_{(3)} (\omega^{ik} p_{,k} \theta_{,j} + \omega_{jk} p^{,k} \theta^{,j}) + H_{(4)} (\omega^{ik} \theta_{,k} p_{,j} + \omega_{jk} \theta^{,k} p^{,j})] + \\
& \frac{\Re}{\rho_m^2} [G_{(10)} d_k^k \theta_{,i} \theta_{,j} \delta_j^i + G_{(11)} d^{k1} \theta_{,i} \theta_{,k} \delta_j^i + G_{(12)} (d^{ik} \theta_{,k} \theta_{,j} + d_{jk} \theta^{,k} \theta^{,i}) + \\
& \quad G_{(13)} d_j^i \theta^{,k} \theta_{,k} + H_{(5)} (\omega^{ik} \theta_{,k} \theta_{,j} + \omega_{jk} \theta^{,k} \theta^{,j})] + \frac{\Re \theta}{\rho_m^3} [G_{(14)} d_k^k p_{,j}^i \delta_j^i + \\
& \quad G_{(15)} d^{k1} p_{,k} \delta_j^i + G_{(16)} (d^{ik} p_{,k} + d_{jk} p^{,k}) + G_{(17)} d_k^k p_{,j}^i + G_{(18)} d_j^i p_{,k}^k + \\
& \quad H_{(6)} (\omega^{ik} p_{,k} + \omega_{jk} p^{,k}) + G_{(19)} d_k^k f_{,i}^i \delta_j^i + G_{(20)} d^{k1} f_{,k}^i \delta_j^i + G_{(21)} (d^{ik} f_{,k}^i + \\
& \quad d_{jk} f^{,k}) + G_{(22)} (d^{ik} f_{,j,k} + d_{jk} f^{,i,k}) + G_{(23)} d_k^k (f_{,j}^i + f_j^i) + G_{(24)} d_j^i f_{,k}^k + \\
& \quad H_{(7)} \omega^{k1} f_{,k}^i \delta_j^i + H_{(8)} (\omega^{ik} f_{,k}^i + \omega_{jk} f^{,k,i}) + H_{(9)} (\omega^{ik} f_{,j,k} + \omega_{jk} f^{,i,k})] + \\
& \frac{\Re}{\rho_m^2} [G_{(25)} d_k^k \theta_{,j}^i \delta_j^i + G_{(26)} d^{k1} \theta_{,k}^i \delta_j^i + G_{(27)} (d^{ik} \theta_{,k}^i + d_{jk} \theta^{,k,i}) + G_{(28)} d_k^k \theta_{,j}^i + \\
& \quad G_{(29)} d_j^i \theta_{,k}^k + H_{(10)} (\omega^{ik} \theta_{,k}^i + \omega_{jk} \theta^{,k,i})] + \frac{1}{\rho_m^2} [K_{(1)} (d_k^k)^2 \delta_j^i + K_{(2)} d_i^i d_k^k d_m^m \delta_j^i + \\
& \quad K_{(3)} d_i^i d_m^m d_k^k \delta_j^i + K_{(4)} d_k^k d_i^i d_j^j + K_{(5)} (d_k^k)^2 d_j^i + K_{(6)} d_k^k d_i^i d_j^j + L_{(1)} \omega_k^i d_m^m d_k^k \delta_j^i + \\
& \quad L_{(2)} (\omega_k^i d_{ij} + d_i^j \omega_{jk}) d^{k1} + M_{(1)} \omega_k^i \omega_l^k d_m^m \delta_j^i + M_{(2)} \omega_k^i \omega_l^m d_k^k \delta_j^i + \\
& \quad M_{(3)} d_k^k \omega^{i1} \omega_{ij} + M_{(4)} (\omega_k^i d_{ij} + d_j^i \omega_{jk}) \omega^{k1} + N_{(1)} \omega_k^i \omega_l^m \omega_m^k \delta_j^i + \\
& \quad N_{(2)} (\omega_k^i \omega_l^k \omega_j^i + \omega_j^k \omega_k^i \omega_l^i)]. \quad (9)
\end{aligned}$$

Here λ is a function of the form (6), and the coefficients $C_{(1)}$, $D_{(1)}$, $E_{(1)}$, $F_{(1)}$, $G_{(1)}$, $H_{(1)}$, $K_{(1)}$, $L_{(1)}$, $M_{(1)}$, $N_{(1)}$ all are dimensionless functions of ρ/ρ_m , θ/θ_0 , $c_{(1)}M_{(1)}$, $c_{(2)}M_{(2)}$, ..., $c_{(k)}M_{(k)}$. The expression for ${}_{(4)}W_j^i$ has also been written down, but since it contains several hundred terms it is too long to reproduce here.

Formula (7) is the Cauchy formula for the viscous stress in the ordinary theory of viscous compressible fluids. A special case of (8) has been derived by Burnett and Chapman-Cowling¹ from the kinetic theory of gases, following a method of formal solution of the Maxwell-Boltzmann equation due in principle to Hilbert and Enskog. Burnett's equations have recently been recommended by Tsien² and Schamberg³ as describing aero-

dynamic phenomena encountered at altitudes of 100,000 to 300,000 feet in the earth's atmosphere. A portion of ${}_{(3)}W_j^i$ has been calculated from the kinetic theory by Chang and Uhlenbeck,⁴ but their result is not available for comparison with (9).

It is interesting to notice that Burnett's expression for W_j^i contains but 7 moduli, while our equations (7) and (8) contain 21, so that to reduce our results to his not only must we discard ${}_{(3)}W_j^i$, ${}_{(4)}W_j^i$, ..., but also we must impose 14 special relations among the coefficients occurring in ${}_{(1)}W_j^i$ and ${}_{(2)}W_j^i$; they are

$$\left. \begin{aligned} 3\lambda + 2\mu &= 0, \\ 3D_{(i)} + 2C_{(i)} &= 0, \quad i = 1, 2, \dots, 6, \\ 3F_{(i)} + 2E_{(i)} &= 0, \quad i = 1, 2, 3, \end{aligned} \right\} \quad (10)$$

$$C_{(1)} = C_{(4)} = -C_{(6)} = E_{(3)} = \frac{1}{2}E_{(4)}. \quad (11)$$

The 10 reductions (10), of which the first is the classical Stokes relation, are necessary and sufficient conditions that

$$p = p_m. \quad (12)$$

The hypotheses of the kinetic theory, at least as employed in the present case, imply this equality of the two pressures at the outset. Since the pressure enters the various moduli λ , μ , $C_{(i)}$, $D_{(i)}$, $E_{(i)}$, $F_{(i)}$ only through the ratio p/p_m it follows that if the special relations (10) be adopted, then these moduli are independent of the pressure, a result familiar in the kinetic theory. It would be more accurate to say that the basic hypotheses of the kinetic theory (at least as employed by the authors mentioned) are such that it is plain from the outset that that theory is not sufficiently general to permit the dependence of the viscosity upon the pressure. The Stokes relation is not now generally accepted except for monatomic gases, nor does it seem probable that the remainder of (10) are correct. The physical meaning of the relations (11) is not apparent, and it does not seem likely they are correct.

The kinetic theory derivation is essentially the computation of successive approximations to a certain special type of solution of the Maxwell-Boltzmann equation, and there is no indication that the expression so derived at a given stage contains all the terms of a given order of magnitude; since the difficulties of calculation enormously increase for each stage, one is at present not able to compare the last terms retained even with the first neglected. The method of derivation presented here enables one to write down any number of terms, and hence suggests dimensionless characteristic numbers which determine the validity of a given approximation. One of these numbers, for example, is

$$\mathcal{V} \equiv \frac{\mu \sqrt{d_j d_i}}{p_m} \quad (13)$$

If $\mathcal{V} \ll 1$ no terms free of thermodynamic gradients need be retained except those whose coefficients are λ and μ , provided the coefficients $E_{(i)}$ and $F_{(i)}$ be not too large.

From the formula (8) may be deduced a rather startling consequence. In the case when the gas is in thermodynamic equilibrium and suffering no deformation whatever, but experiencing a uniform angular velocity ω about the z -axis, we obtain

$$\begin{aligned} W_{xx} = W_{yy} &= -\frac{2\omega^2\mu^2}{\rho_m} (F_{(3)} + E_{(3)}), \\ W_{zz} &= -\frac{2\omega^2\mu^2}{\rho_m} F_{(3)}. \end{aligned} \quad (14)$$

Thus a mass of fluid completely in equilibrium if set into rotation as a rigid body experiences a state of stress which depends in magnitude upon the viscosity of the fluid. Such an occurrence is most improbable. The elementary concept of a fluid would lead one to expect that the rate of dissipation of energy should be quite independent of the rotation, and that that only d_{ij} and not ω_{ij} should appear the basic postulate II. Indeed, in the present theory ω_{ij} was included only so as to obtain end formulae as general as those of kinetic theory. The result (14), however, which of course may be obtained also from the Burnett and Chapman-Cowling equations, suggests that perhaps there may have been an error in the kinetic theory analysis. The method used by Burnett and Chapman-Cowling does not appear to define uniquely the terms which occur at a given stage of the calculation, and it may be possible that in their result the terms involving ω_{ij} may be cancelled by terms from some higher order approximation which has not as yet been determined. This certain degree of arbitrariness may also explain the occurrence of the improbable relations (11) in Burnett's formula, since they might possibly be negated by as yet undiscovered higher order approximations. These remarks are offered only as suggestions. I have not repeated the kinetic theory calculations, which seem formidable. In any case, if ω_{ij} does not appear in Φ at all the end formulae are very greatly simplified, for then

$$F_{(3)} = E_{(3)} = E_{(4)} = H_{(1)} = L_{(1)} = M_{(1)} = N_{(1)} = 0. \quad (15)$$

The analysis given here may easily be generalized so as to include incompressible fluids, for which the definition (1) for the pressure fails, and the pressure must be regarded as a primitive variable.

The preceding considerations are extracted from "A New Definition of a Fluid," shortly to be submitted to the *Journal of Mathematics and Physics*.

* This investigation was carried out under project ONR 45-47 from the Office of Naval Research to the Naval Ordnance Laboratory.

¹ Chapman, S., and Cowling, T. G., *The Mathematical Theory of Non-Uniform Gases*, Cambridge, 1939, see § 15.3 and § 15.4.

² Tsien, H. S., "Superaerodynamics, Mechanics of Rarefied Gases," *J. Aero. Sci.*, **13**, 653-664 (1946).

³ Schamberg, R., *The Fundamental Differential Equations and the Boundary Conditions for High Speed Slip-Flow, and Their Application to Several Specific Problems*, thesis, Calif. Inst. Tech., 1947

⁴ Chang, C. S. Wang, and Uhlenbeck, G. E., *On the Transport Phenomena in Rarified Gases*, Applied Physics Lab. report No. APL/JHU CM-443, 1948.

ERRATA

In the article, "Multiply Valued Harmonic Functions. Green's Theorem," by G. C. Evans, these PROCEEDINGS, **33**, 270-275 (1947), Lemma 1, line 3, replace "each" by " $\mu(e)$;" and Lemma 2, line 3, replace "lower semi-continuous" by "its limit inferior for approach from T."

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- Slater, John Clarke**, 1932 (3), Massachusetts Institute of Technology, Cambridge 39, Mass.
- Slepian, Joseph**, 1941 (4), Westinghouse Electric Corporation, East Pittsburgh, Pa.
- Slichter, Louis Byrne**, 1944 (3), 1446 North Amalfi Drive, Pacific Palisades, Calif.
- Slipher, Vesto Melvin**, 1921 (2), Lowell Observatory, Flagstaff, Ariz.
- Small, Lyndon Frederick**, 1941 (5), National Institute of Health, Bethesda 14, Md.
- Smith, Gilbert Morgan**, 1948 (7), School of Biological Sciences, Stanford University, Stanford University, Calif.
- Smith, Homer William**, 1945 (9), 477 First Avenue, New York 16, N. Y.
- Smith, Lee Irvin**, 1944 (5), School of Chemistry, University of Minnesota, Minneapolis 14, Minn.
- Smith, Paul Althaus**, 1947 (1) Department of Mathematics, Columbia University, New York 27, N. Y.
- Smith, Philip Edward**, 1939 (8), College of Physicians and Surgeons, 630 West 168th Street, New York 32, N. Y.
- Soderberg, Carl Richard**, 1947 (4), Massachusetts Institute of Technology, Cambridge 39, Mass.
- Sonneborn, Tracy Morton**, 1946 (8), Department of Zoology, Indiana University, Bloomington, Ind.
- Spier, Leslie**, 1946 (11), P. O. Box 880, Santa Cruz, Calif.
- Stadie, William Christopher**, 1945 (9), 821 Maloney Clinic, 36th and Spruce Streets, Philadelphia 4, Pa.
- Stadler, Lewis John**, 1938 (7), University of Missouri, Columbia, Mo.
- Stakman, Elvin Charles**, 1934 (7), University Farm, St. Paul 8, Minn.
- Stanley, Wendell Meredith**, 1941 (9), Department of Biochemistry, University of California, Berkeley 4, Calif.
- Stebbins, Joel**, 1920 (2), Washburn Observatory, Madison 6, Wis. After Nov. 1, 1948: Lick Observatory, Mt. Hamilton, Calif.
- Stern, Curt**, 1948 (8), Department of Zoology, University of California, Berkeley 4, Calif.
- Stern, Otto**, 1945 (3), 759 Cragmont Avenue, Berkeley, Calif.
- Stevens, Stanley Smith**, 1946 (12), Department of Psychology, Harvard University, Cambridge 38, Mass.
- Stewart, George Walter**, 1938 (3), State University of Iowa, Iowa City, Iowa.
- Stock, Chester**, 1948 (6), Division of Geological Sciences, California Institute of Technology, Pasadena 4, Calif.
- Stone, Calvin Perry**, 1943 (12), Stanford University, Stanford University, Calif.
- Stone, Marshall Harvey**, 1938 (1), 313 Eckhart Hall, University of Chicago, Chicago 37, Ill.

- Streeter, George Linus**, 1931 (8), Johns Hopkins Medical School, 710 North Washington Street, Baltimore 5, Md.
- Struve, Otto**, 1937 (2), Yerkes Observatory, Williams Bay, Wis.
- Sturtevant, Alfred Henry**, 1930 (8), California Institute of Technology, Pasadena 4, Calif.
- Suits, Chauncey Guy**, 1946 (4), Research Laboratory, General Electric Company, Schenectady 5, N. Y.
- Sumner, James Batcheller**, 1948 (5), New York State College of Agriculture, Laboratory of Enzyme Chemistry, Cornell University, Ithaca, N. Y.
- Sverdrup, Harald Ulrik**, 1945 (3), Norsk Polarinstitut, Observatoriegte. 1, Oslo, Norway
- Swanton, John Reed**, 1932 (11), 22 George Street, Newton 58, Mass.
- Taliaferro, William Hay**, 1940 (8), University of Chicago, Chicago 37, Ill.
- Tate, John Torrence**, 1942 (3), University of Minnesota, Minneapolis 14, Minn.
- Teller, Edward**, 1948 (3), Institute for Nuclear Studies, University of Chicago, Chicago 37, Ill.
- Terman, Frederick Emmons**, 1946 (4), School of Engineering, Stanford University, Stanford University, Calif.
- Terman, Lewis, Madison**, 1928 (12), 761 Dolores Street, Stanford University, Calif.
- Thimann, Kenneth Vivian**, 1948 (7), Harvard University, Biological Laboratories, 16 Divinity Avenue, Cambridge 38, Mass.
- Thom, Charles**, 1937 (7), 207 Grant Street, Port Jefferson, N. Y.
- Thomas, Charles Allen**, 1948 (4), Monsanto Chemical Company, 1700 South Second Street, St. Louis 4, Mo.
- Thomas, Tracy Yerkes**, 1941 (1), Swain Hall, Indiana University, Bloomington, Ind.
- Thorndike, Edward Lee**, 1917 (12), Teachers College, Columbia University, New York 27, N. Y.
- Thurstone, Louis Leon**, 1938 (12), University of Chicago, Chicago 37, Ill.
- Timoshenko, Stephen Prokop**, 1940 (4), Room 262, Engineering Building, Stanford University, Calif.
- Tolman, Edward Chace**, 1937 (12), Department of Psychology, University of California, Berkeley 4, Calif.
- Tolman, Richard Chace**, 1923 (5), California Institute of Technology, Pasadena 4, Calif.
- Tozzer, Alfred Marston**, 1942 (11), Peabody Museum, Harvard University, Cambridge 38, Mass.
- Trumpler, Robert Julius**, 1932 (2), Berkeley Astronomical Department, University of California, Berkeley 4, Calif.
- Tuve, Merle Antony**, 1946 (3), Department of Terrestrial Magnetism, Carnegie Institution of Washington, 5241 Broad Branch Road, N. W., Washington 15, D. C.
- Tyzzer, Ernest Edward**, 1942 (10), 175 Water Street, Wakefield, Mass.

- Urey, Harold Clayton**, 1935 (5), Institute for Nuclear Studies, University of Chicago, Chicago 37, Ill.
- Vandiver, Harry Shultz**, 1934 (1), Department of Applied Mathematics, University of Texas, Austin 12, Tex.
- Van Niel, Cornelis Bernardus**, 1945 (7), Hopkins Marine Station of Stanford University, Pacific Grove, Calif.
- Van Slyke, Donald Dexter**, 1921 (9), Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, N. Y.
- Van Vleck, John Hasbrouck**, 1935 (3), Harvard University, Cambridge 38, Mass.
- Vaughan, Thomas Wayland**, 1921 (6), 3333 P Street, N. W., Washington 7, D. C.
- Veblen, Oswald**, 1919 (1), The Institute for Advanced Study, Princeton, N. J.
- Vickery, Hubert Bradford**, 1943 (9), Connecticut Agricultural Experiment Station, New Haven 4, Conn.
- von Karman, Theodore**, 1938 (4), California Institute of Technology, Pasadena 4, Calif.
- von Neumann, John**, 1937 (1), The Institute for Advanced Study, Princeton, N. J.
- Waksman, Selman Abraham**, 1942 (7), Agricultural Experiment Station, New Brunswick, N. J.
- Walker, John Charles**, 1945 (7), 206 Horticulture Building, University of Wisconsin, Madison 6, Wis.
- Walsh, Joseph Leonard**, 1936 (1), Harvard University, Cambridge 38, Mass.
- Webster, David Locke**, 1923 (3), Physics Department, Room 385, Stanford University, Stanford University, Calif.
- Weiss, Paul Alfred**, 1947 (8), Department of Zoology, University of Chicago, Chicago 37, Ill.
- Went, Frits Warmolt**, 1947 (7), California Institute of Technology, Pasadena 4, Calif.
- Werkman, Chester Hamlin**, 1946 (9), Department of Bacteriology, Iowa State College, Ames, Iowa
- Wetmore, Alexander**, 1945 (8), Smithsonian Institution, Washington 25, D. C.
- Wever, Ernest Glen**, 1940 (12), Princeton University, Princeton, N. J.
- Weyl, Claus Hugo Hermann**, 1940 (1), The Institute for Advanced Study, Princeton, N. J.
- Whipple, George Hoyt**, 1929 (10), School of Medicine, University of Rochester, 260 Crittenden Boulevard, Rochester 7, N. Y.
- Whitehead, John Boswell**, 1932 (4), Johns Hopkins University, Baltimore 18, Md.
- Whitney, Hassler**, 1945 (1), Sudbury Road, Weston 93, Mass.
- Whitney, Willis Rodney**, 1917 (5), General Electric Company, Schenectady 5, N. Y.
- Wigner, Eugene Paul**, 1945 (3), 8 Ober Road, Princeton, N. J.

- Williams, Robert R.**, 1945 (5), 297 Summit Avenue, Summit, N. J.
Williams, Roger John, 1946 (5), Biochemical Institute, University of Texas, Austin 12, Tex.
Willier, Benjamin Harrison, 1945 (8), Department of Biology, Johns Hopkins University, Baltimore 18, Md.
Willis, Bailey, 1920 (6), Box 1365, Stanford University, Calif.
Wilson, Edgar Bright, Jr., 1947 (5), Harvard University, Department of Chemistry, 12 Oxford Street, Cambridge 38, Mass.
Wilson, Edwin Bidwell, 1919 (3), Harvard School of Public Health, 695 Huntington Avenue, Boston 15, Mass.
Wilson, Robert Erastus, 1947 (4), 910 South Michigan Avenue, Chicago 80, Ill.
Wislocki, George Bernays, 1941 (8), Harvard Medical School, 25 Shattuck Street, Boston 15, Mass.
Wolbach, Simeon Burt, 1938 (10), Department of Pathology, Children's Hospital, 300 Longwood Avenue, Boston 15, Mass.
Wood, Robert Williams, 1912 (3), Johns Hopkins University, Baltimore 18, Md.
Woodring, Wendell Phillips, 1946 (6), United States Geological Survey, Washington 25, D. C.
Woodworth, Robert Sessions, 1921 (12), Columbia University, New York 27, N. Y.
Wright, Frederick Eugene, 1923 (6), 2134 Wyoming Avenue, N. W., Washington 8, D. C.
Wright, Sewall Green, 1934 (8), Department of Zoology, University of Chicago, Chicago 37, Ill.
Wright, William Hammond, 1922 (2), 60 North Keeble Avenue, San Jose 11, Calif.
Yerkes, Robert Mearns, 1923 (12), Yale University School of Medicine, 333 Cedar Street, New Haven, Conn.
Yost, Don Merlin Lee, 1944 (5), California Institute of Technology, Pasadena 4, Calif.
Zariski, Oscar, 1944 (1), Department of Mathematics, Harvard University, Cambridge 38, Mass.
Zworykin, Vladimir Kosma, 1943 (4), 103 Battle Road, Princeton, N. J.

Number of Members July 1, 1948: 430

MEMBERS EMERITI

- Benedict, Francis Gano**, 1914, Machiasport, Me.
Dewey, John, 1910, 1158 Fifth Avenue, New York 29, N. Y.
Dickson, Leonard Eugene, 1913, Route #2, Joliet, Ill.
Howard, Leland Ossian, 1916, Bureau of Entomology, U. S. Department, of Agriculture, Washington 25, D. C.
Stratton, George Malcolm, 1928, University of California, Berkeley 4, Calif.

FOREIGN ASSOCIATES

The letter in parentheses following the year of election indicates the field of scientific research in which the foreign associate was working at the time of his election, as follows:

- | | |
|------------------------------|---------------------------------|
| (A) Mathematics | (G) Botany |
| (B) Astronomy | (H) Zoology and Anatomy |
| (C) Physics | (I) Physiology and Biochemistry |
| (D) Engineering | (J) Pathology and Bacteriology |
| (E) Chemistry | (K) Anthropology and Psychology |
| (F) Geology and Paleontology | |

- Adrian, Edgar Douglas**, 1941 (I), Trinity College, Cambridge, England
- Alexandroff, Paul A.**, 1947 (A), Mathematical Institute of the Academy of Sciences of the U.S.S.R., Bolshaya Kalushskaya 19, Moscow, U.S.S.R.
- Bailey, Sir Edward**, 1944 (F), 19 Greenhill Gardens, Edinburgh 10, Scotland
- Bartlett, Frederic Charles**, 1947 (K), University of Cambridge, The Psychological Laboratory, Downing Place, Cambridge, England
- Bjerknes, V. (F. K.)**, 1934 (F), The University, Oslo, Norway
- Bohr, Niels**, 1925 (C), The Institute for Advanced Study, Princeton, N. J. (U.S.A.)
- Bordet, Jules**, 1935 (I), Pasteur Institute, Rue du Remorqueur, 28, Brussels, Belgium
- Bragg, Sir William Lawrence**, 1945 (C), Cavendish Laboratory, The University, Cambridge, England
- de Broglie, Prince Louis**, 1948 (C), 94 Rue Perronet, Neuilly-sur-Seine, France
- Caso, Alfonso**, 1943 (K), Secretaria de Bienes Nacionales e Inspeccion Administrativa, Mexico, D. F.
- Chapman, Sydney**, 1946 (A), Letters: Queen's College, Oxford; Printed matter: The Mathematical Institute, Oxford, England
- Dale, Sir Henry Hallett**, 1940 (I), The Wellcome Trust, 28 Portman Square, London, W.1, England
- Debye, Peter**, 1931* (C), Baker Laboratory, Cornell University, Ithaca, N. Y. (U.S.A.)
- Einstein, Albert**, 1922† (C), The Institute for Advanced Study, Princeton, N. J. (U.S.A.)
- Fisher, Ronald Aylmer**, 1948 (H), Department of Genetics, University of Cambridge, 44 Storey's Way, Cambridge, England
- Hadamard, Jacques**, 1926 (A), 12, rue Emile Faguet, Paris XIV, France

* Dr. Debye became a naturalized citizen in 1946 and was elected a member of the Academy in 1947.

† Dr. Einstein became a naturalized American citizen in 1941 and was elected a member of the Academy in 1942.

If a foreign associate becomes a member of the Academy his name is not counted in the limit of 50 foreign associates.

- Hardy, Godfrey Harold**, 1927 (A), Trinity College, Cambridge, England
Helland-Hansen, Björn, 1947 (F), Chr. Michelsens Institutt for Videnskap, Bergen, Norway
Hill, Archibald Vivian, 1941 (I), 16 Bishopwood Road, Highgate, London, N.6, England
Hill, James Peter, 1940 (H), Kanimbla, Dollis Avenue, London N.3, England
Houssay, Bernardo Alberto, 1940 (I), Viamonte 2790, Buenos Aires, Argentina
Janet, Pierre, 1938 (K), 54, rue de Varenne, Paris VII, France
Jeffreys, Harold, 1945 (B), St. John's College, Cambridge, England
Jones, Sir Harold Spencer, 1943 (B), Royal Observatory, Greenwich, London, S.E.10, England
Kapitza, Peter Leonidovich, 1946 (C), Institute for Physical Problems, Academy of Sciences of the U.S.S.R., Moscow, U.S.S.R.
Karrer, Paul, 1945 (E), University of Zurich, Zurich, Switzerland
Keith, Sir Arthur, 1941 (I, K), Buckston Browne Farm, Downe, Farnborough, Kent, England
Krogh, August, 1937 (H), The Laboratory, Söbredde, Gentofte, Denmark
Levi, Giuseppe, 1940 (H), Istituto di Anatomia Umana, Corso Massimo D'Azeglio, 52, Turin, Italy
Limm, Robert K. S., 1942 (I), Army Medical Administration, Municipal Government, Shanghai, China
Linderstrøm-Lang, Kaj Ulrik, 1947 (E), Chemical Division, Carlsberg Laboratory, Copenhagen, Denmark
Robinson, Sir Robert, 1934 (E), Dyson Perrins Laboratory, South Parks Road, Oxford, England
Ruzicka, Leopold, 1944 (E), Department of Organic Chemistry, Institute of Technology, Zurich, Switzerland
Sherrington, Sir Charles Scott, 1924 (I), Gonville and Caius College, Cambridge, England
Sommerfeld, Arnold, 1929 (C), Dunant-strasse 6, Munich 23, Germany
Southwell, Richard Vynne, 1943 (D), Imperial College of Science and Technology, South Kensington, London, S.W.7, England
Svedberg, The, 1945 (E), Fysikalisk-Kemiska Institutionen, University of Uppsala, Uppsala, Sweden
Taylor, Sir Geoffrey I., 1945 (A), Trinity College, Cambridge, England
Vallee-Poussin, C. de la, 1929 (A), University of Louvain, Louvain, Belgium
Van der Bijl, Hendrik Johannes, 1943 (D), 722 Escom House, Rissik Street, Johannesburg, South Africa
Vening Meinesz, Felix Andries, 1939 (F), Potgieterlaan 5, Amersfoort, Holland
Watson, D. M. S., 1938 (H), University College, Gower Street, London, W.C.1, England
Wieland, Heinrich, 1932 (E), Sophienstrasse 9, Munich 2 NW, Germany

Number of Foreign Associates July 1, 1948: 41.

SECTIONS

(1) *Mathematics*—26 members

von Neumann, John, <i>Chairman</i> (1949)	Evans, G. C.	Smith, P. A.
Albert, A. A.	Kasner, Edward	Stone, M. H.
Alexander, J. W.	Lefschetz, Solomon	Thomas, T. Y.
Bell, E. T.	McShane, E. J.	Vandiver, H. S.
Bliss, G. A.	Miller, G. A.	Veblen, Oswald
Coble, A. B.	Moore, R. L.	Walsh, J. L.
Douglas, Jesse	Morse, Marston	Weyl, Hermann
Eisenhart, L. P.	Murnaghan, F. D.	Whitney, Hassler
	Ritt, J. F.	Zariski, Oscar

(2) *Astronomy*—26 members

Mitchell, S. A., <i>Chair-</i> <i>man</i> (1950)	Hubble, E. P.	Ross, F. E.
Abbot, C. G.	Joy, A. H.	Russell, H. N.
Adams, W. S.	King, A. S.	Seares, F. H.
Aitken, R. G.	Leuschner, A. O.	Shapley, Harlow
Anderson, J. A.	Menzel, D. H.	Slipher, V. M.
Babcock, H. D.	Merrill, P. W.	Stebbins, Joel
Bowen, I. S.	Moore, J. H.	Struve, Otto
Fleming, J. A.	Moulton, F. R.	Trumpler, R. J.
	Nicholson, S. B.	Wright, W. H.

(3) *Physics*—60 members

Beams, J. W., <i>Chair-</i> <i>man</i> (1951)	Davisson, C. J.	Oppenheimer, J. R.
Allison, S. K.	Dempster, A. J.	Pierce, G. W.
Alvarez, L. W.	DuBridge, L. A.	Piggot, C. S.
Anderson, C. D.	Dunning, J. R.	Rabi, I. I.
Bacher, R. F.	Einstein, Albert	Reichelderfer, F. W.
Bainbridge, K. T.	Epstein, P. S.	Rossby, C.-G.
Berkner, L. V.	Fermi, Enrico	Saunders, F. A.
Bethe, H. A.	Franck, James	Slater, J. C.
Birge, R. T.	Goudsmit, S. A.	Slichter, L. B.
Bjerknes, Jacob	Houston, W. V.	Stern, Otto
Bloch, Felix	Hull, A. W.	Stewart, G. W.
Breit, Gregory	Ives, H. E.	Sverdrup, H. U.
Bridgman, P. W.	Kemble, E. C.	Tate, J. T.
Coblentz, W. W.	Lauritsen, C. C.	Teller, Edward
Compton, A. H.	Lawrence, E. O.	Tuve, M. A.
Compton, K. T.	Lyman, Theodore	Van Vleck, J. H.
Condon, E. U.	McMillan, E. M.	Webster, D. L.
Coolidge, W. D.	Mason, Max	Wigner, E. P.
Crew, Henry	Millikan, R. A.	Wilson, Edwin B.
Davis, Bergen	Mulliken, R. S.	Wood, R. W.

(4) *Engineering*—35 members

Dryden, H. L., <i>Chairman</i> (1950)	Foote, P. D.	Loomis, A. L.
Adams, C. A.	Gilliland, E. R.	Merica, P. D.
Briggs, L. J.	Herty, C. H., Jr.	Slepian, Joseph
Buckley, O. E.	Hoover, Herbert	Soderberg, C. R.
Bush, Vannevar	Hovgaard, William	Suits, C. G.
Cochrane, E. L.	Hunsaker, J. C.	Terman, F. E.
Cottrell, F. G.	Jeffries, Zay	Thomas, C. A.
Curne, G. O., Jr.	Jewett, F. B.	Timoshenko, Stephen
Dunn, Gano	Kelly, M. J.	von Karman, T.
Durand, W. F.	Kettering, C. F.	Whitehead, J. B.
Fletcher, Harvey	Lewis, G. W.	Wilson, R. E.
	Lewis, W. K.	Zworykin, V. K.

(5) *Chemistry*—58 members

Latimer, W. M., <i>Chairman</i> (1950)	Gilman, Henry	Marvel, C. S.
Adams, L. H.	Hammett, L. P.	Mayer, J. E.
Adams, Roger	Harkins, W. D.	Noyes, W. A., Jr.
Adkins, Homer	Hildebrand, J. H.	Onsager, Lars
Bachmann, W. E.	Hudson, C. S.	Pauling, Linus
Bancroft, W. D.	Hulett, G. A.	Rodebush, W. H.
Bartlett, P. D.	Ipatieff, V. N.	Scatchard, George
Baxter, G. P.	Jacobs, W. A.	Schlesinger, H. I.
Bogert, M. T.	Johnson, J. R.	Seaborg, G. T.
Bolton, E. K.	Keyes, F. G.	Small, L. F.
Conant, J. B.	Kharasch, M. S.	Smith, L. I.
Cope, A. C.	Kirkwood, J. G.	Sumner, J. B.
Daniels, Farrington	Kistiakowsky, G. B.	Tolman, R. C.
Debye, Peter	Kraus, C. A.	Urey, H. C.
Eyring, Henry	La Mer, V. K.	Whitney, W. R.
Fieser, L. F.	Lamb, A. B.	Williams, Robert R.
Folkers, Karl	Langmuir, Irving	Williams, Roger J.
Fuson, R. C.	Lind, S. C.	Wilson, E. Bright, Jr.
Giauque, W. F.	Longworth, L. G.	Yost, D. M.
	MacInnes, D. A.	

(6) *Geology*—38 members

Rubey, W. W., <i>Chairman</i> (1951)	Buddington, A. F.	Gregory, W. K.
Allen, E. T.	Byerly, Perry	Gutenberg, Beno
Berkey, C. P.	Chaney, R. W.	Hewett, D. F.
Blackwelder, Eliot	Cross, Whitman	Kelley, W. P.
Bowen, N. L.	Daly, R. A.	Knopf, Adolph
Bowman, Isaiah	Day, A. L.	Larsen, E. S., Jr.
Bradley, W. H.	Dunbar, C. O.	Lawson, A. C.
Bucher, W. H.	Ewing, Maurice	Leith, C. K.
	Gilluly, James	Longwell, C. R.

Macelwane, J. B.
Mead, W. J.
Mendenhall, W. C.
Palache, Charles

Reeside, J. B., Jr.
Ruedemann, Rudolf
Simpson, G. G.
Stock, Chester

Vaughan, T. W.
Willis, Bailey
Woodring, W. P.
Wright, F. E.

(7) *Botany*—35 members

Cleland, R. E., *Chairman* (1950)
Allen, C. E.
Babcock, E. B.
Bailey, I. W.
Bailey, L. H.
Beadle, G. W.
Blakeslee, A. F.
Brink, R. A.
Campbell, D. H.
Chandler, W. H.
Couch, J. N.

Dodge, B. O.
Duggar, B. M.
Fernald, M. L.
Fred, E. B.
Hoagland, D. R.
Jones, D. F.
Kunkel, L. O.
McClintock, Barbara
Mangelsdorf, P. C.
Merrill, E. D.
Osterhout, W. J. V.
Rhoades, M. M.

Robbins, W. J.
Sax, Karl
Sinnott, E. W.
Smith, Gilbert M.
Stadler, L. J.
Stakman, E. C.
Thimann, K. V.
Thom, Charles
Van Niel, C. B.
Waksman, S. A.
Walker, J. C.
Went, F. W.

(8) *Zoology and Anatomy*—38 members

Wright, Sewall, *Chairman*, (1949)
Bigelow, H. B.
Castle, W. E.
Child, C. M.
Conklin, E. G.
Corner, G. W.
Danforth, C. H.
Demerec, Milislav
Detwiler, S. R.
Dobzhansky, Theodosius
Dunn, L. C.
Goldschmidt, R. B.

Harrison, R. G.
Hartman, C. G.
Harvey, E. N.
Herrick, C. J.
Hisaw, F. L.
Jacobs, M. H.
Lewis, W. H.
Metz, C. W.
Moore, Carl R.
Muller, H. J.
Painter, T. S.
Parker, G. H.
Patterson, J. T.

Riddle, Oscar
Romer, A. S.
Schmitt, F. O.
Smith, P. E.
Sonneborn, T. M.
Stern, Curt
Streeter, G. L.
Sturtevant, A. H.
Taliaferro, W. H.
Weiss, Paul
Wetmore, Alexander
Willier, B. H.
Wislocki, G. B.

(9) *Physiology and Biochemistry*—45 members

Doisy, E. A., *Chairman* (1951)
Anderson, R. J.
Ball, E. G.
Bard, Philip
Bronk, D. W.
Carlson, A. J.
Clark, W. M.
Clarke, H. T.
Cohn, E. J.
Cori, Carl F.

Cori, Gerty T.
Davis, Hallowell
DuBois, E. F.
du Vigneaud, Vincent
Elvehjem, C. A.
Erlanger, Joseph
Evans, H. M.
Fenn, W. O.
Forbes, Alexander
Gasser, H. S.
Hart, E. B.

Hartline, H. K.
Hastings, A. B.
Link, K. P.
Loeb, R. F.
Long, C. N. H.
McCollum, E. V.
MacNider, W. deB.
Marshall, E. K., Jr.
Maynard, L. A.
Meek, W. J.
Michaelis, Leonor

Northrop, J. H.	Rose, W. C.	Stadie, W. C.
Oncley, J. L.	Shaffer, P. A.	Stanley, W. M.
Peters, J. P.	Sherman, H. C.	Van Slyke, D. D.
Richards, A. N.	Smith, Homer W.	Vickery, H. B.
		Werkman, C. H.

(10) *Pathology and Bacteriology*—37 members

Dóchez, A. R., <i>Chairman</i> (1951)	Goodpasture, E. W.	Murphy, J. B.
Addis, Thomas	Graham, E. A.	Novy, F. G.
Armstrong, Charles	Heidelberger, Michael	Opie, E. L.
Avery, O. T.	Hektoen, Ludvig	Paul, John R.
Blake, F. G.	Horsfall, F. L., Jr.	Rivers, T. M.
Blalock, Alfred	Kelser, R. A.	Robertson, O. H.
Cannon, P. R.	Little, C. C.	Rous, Peyton
Castle, W. B.	Loeb, Leo	Sabin, Florence R.
Cole, Rufus	Long, E. R.	Shope, R. E.
Dubos, R. J.	Longcope, W. T.	Tyzzar, E. E.
Francis, Thomas, Jr.	Meyer, K. F.	Whipple, G. H.
Gamble, J. L.	Minot, G. R.	Wolbach, S. B.
	Mueller, J. Howard	

(11) *Anthropology*—9 members

Linton, Ralph, <i>Chairman</i> (1951)	Kidder, A. V.	Spier, Leslie
Hooton, E. A.	Kroeber, A. L.	Swanton, J. R.
	Lowie, R. H.	Tozzer, A. M.
	Schultz, A. H.	

(12) *Psychology*—23 members

Boring, E. G., <i>Chairman</i> (1950)	Hunter, W. S.	Stone, C. P.
Angell, J. R.	Köhler, Wolfgang	Terman, L. M.
Carmichael, Leonard	Lashley, K. S.	Thorndike, E. L.
Gesell, Arnold	Miles, W. R.	Thurstone, L. L.
Graham, C. H.	Pillsbury, W. B.	Tolman, E. C.
Hilgard, E. R.	Richter, C. P.	Wever, E. G.
Hull, C. L.	Seashore, C. E.	Woodworth, R. S.
	Stevens, S. S.	Yerkes, R. M.

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29 members

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*RADIAL OSCILLATIONS OF THE LIMITING MODELS OF POLY-
TROPIC GAS SPHERES*

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A gas sphere is said to be in polytropic equilibrium if the relationship between the pressure P and density ρ at any point in its interior is expressed as

$$P = K\rho^{1+1/n} \quad (1)$$

where K and n are constants. If we introduce, as usual, the non-dimensional variables θ and ξ related with the density ρ at a distance r from the center by

$$r = \left[\frac{(1+n)P_c}{4\pi G\rho_c^2} \right]^{1/2} \xi \text{ and } \rho = \rho_c \theta^n, \quad (2)$$

where the subscript c denotes the central values of the respective quantities and G stands for the gravitational constant, the new function θ describing the distribution of density in the interior of a polytropic gas sphere under the influence of its own gravity is governed by Emden's well-known equation

$$\frac{1}{\xi^2} \frac{d}{d\xi} \left(\xi^2 \frac{d\theta}{d\xi} \right) + \theta^n = 0. \quad (3)$$

If the mass of a polytrope is to be finite, it can be shown that $0 \leq n \leq 5$. For both limiting cases, equation (2) admits of a solution in closed form: namely, if $n = 0$, the solution of (3) which is regular at the center becomes

$$\theta = 1 - \frac{1}{6} \xi^2, \quad (4)$$

whereas if $n = 5$,

$$\theta = \left(1 + \frac{1}{3} \xi^2\right)^{-1/2}. \quad (5)$$

The former case refers to a gas sphere the density of which is constant throughout the interior; the latter, to a sphere of finite central density but of infinite radius and therefore vanishing mean density.

The object of the present paper is to investigate what happens when a polytropic gas sphere characterized by $n = 0$ or $n = 5$ is disturbed from its state of equilibrium by an instantaneous impulse in such a way that a small purely radial motion results. If the corresponding displacement $\delta\xi$ of ξ occurs adiabatically, and is so small that squares and higher powers of its amplitude can be ignored, Eddington¹ has shown that the Lagrangian hydrodynamical equations can be reduced to the following second-order differential equation

$$\frac{d^2 f}{d\xi^2} + \frac{4 - \mu}{\xi} \frac{df}{d\xi} + \left\{ \frac{\omega^2}{\theta} - \frac{\alpha\mu}{\xi^2} \right\} f = 0, \quad (6)$$

where $f \equiv \delta\xi/\xi$ and

$$\begin{aligned} \mu &= -(n+1) \frac{\xi}{\theta} \frac{d\theta}{d\xi}, \\ \alpha &= 3 - \frac{4}{\gamma}, \\ \omega^2 &= \frac{(n+1)\nu^2}{4\pi G\gamma\rho_c}, \end{aligned} \quad (7)$$

$2\pi/\nu$ being the period of the respective oscillation and γ , the ratio of specific heats. The boundary conditions of our problem which require that there be no displacement at the center and no variation of pressure on the surface are formally equivalent to a requirement that equation (6) have regular integrals that are finite for $0 \leq \xi \leq \xi_1$, where ξ_1 denotes the first zero of the respective Emden function.

Polytrope $n = 0$.—Consider first a compressible gas sphere in which the undisturbed density is constant and $\xi_1 = \sqrt{6}$. Setting $\xi/\xi_1 = x$ and making use of (4) we find that $\mu = 2x^2/(1-x^2)$ and equation (6) assumes the form

$$(1-x^2) \frac{d^2 f}{dx^2} + \frac{4-x^2}{x} \frac{df}{dx} + Jf = 0, \quad (8)$$

where we have abbreviated

$$J = 6\omega^2 - 2\alpha. \quad (9)$$

A substitution $x^2 = y$ transforms (8) into a hypergeometric equation

$$y(1-y) \frac{d^2 f}{dy^2} + [c - (a+b+1)y] \frac{df}{dy} - abf = 0, \quad (10)$$

where

$$a, b = \frac{5}{4} \pm \sqrt{\left(\frac{5}{4}\right)^2 + \frac{1}{4} J} \quad (11)$$

and $c = 5/2$.

The particular solution of (10) which remains regular at the origin is

$$f = AF\left(a, b, \frac{5}{2}, x^2\right), \quad (12)$$

where A is an arbitrary constant. The second requirement of our boundary conditions, that f be finite for $x^2 = 1$, discloses that the hypergeometric series on the right-hand side of (12) must either converge or—if divergent—must be terminating. In our present problem $a + b = c$, in which case the hypergeometric series is known to diverge for $x = 1$.² Hence, if (12) is to represent a physically admissible solution of our problem, the series on the right-hand side of (12) must reduce to a polynomial—which means that a or b must be equal to some negative integer. If we assume that $b = -j$ (an assumption of $a = -j$ would lead to exactly the same consequences), where j is zero or a positive integer, it follows from (11) that

$$J = 2j(2j + 5). \quad (13)$$

Therefore, a homogeneous gas sphere, disturbed slightly from its state of equilibrium, is found to be able to oscillate radially, not in any frequency, but only in discrete frequencies given by

$$\nu^2 = \frac{4}{3} \pi G \rho_c \gamma \{j(2j + 5) + \alpha\}, \quad (14)$$

which are real provided that $J + 2\alpha > 0$, i.e., that

$$\gamma > \frac{4}{j(2j + 5) + 3}. \quad (15)$$

The corresponding eigenfunctions (12) of the radial motion reduce them to the Jacobi polynomials³

$$f = AG_1\left(\frac{5}{2}, \frac{5}{2}, x^2\right), \quad (16)$$

while the corresponding variation δP of pressure P accompanying the radial oscillation is given by

$$\frac{\delta P}{P} = -\frac{\gamma}{x^2} \frac{d}{dx} (x^3 f) = -3\gamma AG_j \left(\frac{5}{2}, \frac{3}{2}, x^2 \right). \quad (17)$$

It should be mentioned that the discrete nature of the frequency spectrum of radial oscillations of a homogeneous configuration of compressible gas was previously pointed out by Sterne;⁴ in point of fact, its fundamental period (corresponding to $j = 0$) was first found by Miller.⁵ Both these investigators failed, however, to recognize the hypergeometric character of the equation (8), or the fact that the proof of the discrete nature of the spectrum is based on the divergence of a hypergeometric series of unit radius, and that the respective eigenfunctions of the oscillation in radius or pressure are Jacobi polynomials.

Polytrope $n = 5$.—Let us now turn to the other limiting case of the polytropic family of models—that involving an infinite degree of central condensation in which $n = 5$. From the astrophysical point of view, this case is of much greater interest than a homogeneous configuration since the internal density concentrations in real stars are known to be high and, therefore, the properties of a polytrope $n = 5$ may, in certain respects, come close to those of actual stars.

In order to investigate the possibility of small radial oscillations of a gas sphere built up according to this model, we return to Schuster's solution (5) of Emden's equation (3) for $n = 5$ and introduce a new independent variable τ defined by

$$\xi = \sqrt{3} \tan \tau. \quad (18)$$

From this it is easily demonstrated that $\theta = \cos \tau$ and $\mu = 6 \sin^2 \tau$. Assuming moreover,

$$f = \frac{v(\tau)}{\sin^2 \tau \cos^2 \tau}, \quad (19)$$

Eddington's equation (6) governing small radial oscillations of polytropic stars takes the explicit form

$$\frac{d^2 v}{d\tau^2} + 2 \left\{ 8 - \frac{1}{\sin^2 \tau} + \frac{2}{\cos^2 \tau} \left(\frac{6}{\gamma} - 5 \right) + \frac{3\omega^2}{2 \cos^5 \tau} \right\} v = 0. \quad (20)$$

Before proceeding with the solution of this equation, let us make more precise the physical significance of the model we are considering. The polytrope $n = 5$ represents a gas sphere of finite mass and central density, but of infinite extent. With certain precautions such a configuration can, however, be converted into one of finite mass and dimensions, but of infinite central density. In more specific terms, the mass m and radius r of the polytrope $n = 5$ are known⁶ to be

$$\left. \begin{aligned} m(\tau) &= 4\sqrt{3} \pi \left(\frac{3K}{2\pi G} \right)^{1/2} \rho_c^{-1/2} \sin^3 \tau, \\ r(\tau) &= 3 \left(\frac{K}{2\pi G} \right)^{1/2} \rho_c^{-1/2} \tan \tau, \end{aligned} \right\} \quad (21)$$

with boundary at $\tau = 90^\circ$. From these equations it is obvious that the only way to obtain finite dimensions of the whole configuration for $K \neq 0$ is to make ρ_c infinitely large. A polytrope $n = 5$ is, however, seen to possess the curious feature that its mass tends toward zero, for finite values of K , when its central density increases beyond any limit. The mass can, therefore, remain finite only provided that ρ_c and K both approach infinity in such a way that

$$K^{1/2}/\rho_c^{1/2} = \text{constant}. \quad (22)$$

The radius r then will, by definition, be a finite limit of the indefinite product $\rho_c^{1/2} \cos 90^\circ$; the reader can easily verify that this definition is sufficient to make the pressure vanish at the boundary of our configuration.

If the foregoing conditions are met, it is apparent that an assumption of infinite central density can effect a homologous transformation of the polytrope $n = 5$ into a configuration of finite dimensions, the whole mass of which is confined to its center and surrounded by an envelope of negligible weight, in which the density falls off as the inverse fifth power of the distance from the center. Equations (7) make it evident, however, that, under these circumstances, *the value of ω^2 is found to be zero for any finite frequency ν of free radial oscillation of the respective configuration.* In virtue of this fact, the term of the right-hand side of (20) multiplied by ω^2 drops off, and the remaining equation proves again to be one of the hypergeometric type.

Changing over from τ to a new independent variable $x = \sin^2 \tau$ and taking

$$v(\tau) = \eta(x) \sin^m \tau \cos^n \tau, \quad (23)$$

where

$$m(m-1) = 2, \quad (24)$$

$$n(n-1) = 4(5-6/\gamma) \quad (25)$$

equation (20) can be reduced to

$$x(1-x) \frac{d^2 \eta}{dx^2} + [c - (a+b+1)x] \frac{d\eta}{dx} - ab\eta = 0, \quad (26)$$

where now

$$\left. \begin{aligned} a &= \frac{1}{2}(m + n + 4), \\ b &= \frac{1}{2}(m + n - 4), \\ c &= \frac{1}{2} + m, \end{aligned} \right\} \quad (27)$$

and combining its general solution with (19) and (23) it follows that

$$f = \sin^{m-2} \tau \cos^{n-2} \tau \{ A F(a, b, c, x) + B x^{1-c} F(a+1-c, b+1-c, 2-c, x) \}, \quad (28)$$

where A and B are integration constants.

The boundary conditions of our problem require f to remain finite for $x = 0$ and 1. The condition at the origin asserts at once that $B = 0$ and that, of the two roots of equation (24), only $m = 2$ is admissible; for then $f(0) = A$ which, without the loss of generality, can be set equal to one. This leaves us with

$$f = (1-x)^b F\left(a, b, \frac{5}{2}, x\right) \quad (29)$$

as a possible solution of our physical problem, where $a = \frac{1}{2}(n+6)$ and $b = \frac{1}{2}(n-2)$, n being a root of equation (25) whose value depends on γ .

If the pressure is to remain constant over the free surface of our radially oscillating configuration, f must retain a finite value at $x = 1$. The hypergeometric series on the right-hand side of equation (29) will again diverge or converge for $x = 1$ as $a + b \geq c$, i.e., as n is greater or smaller than one half. An inspection of equation (25) reveals that, for any value of $\gamma > 32/27$, the equation admits indeed of a pair of roots one of which is always less than 0.5, so that for every value of γ greater than this limit a value of n can be found which will make our hypergeometric series converge. However, if $n < 0.5$, then $b < -0.75$, and therefore the binomial $(1-x)^b$ factoring the hypergeometric series on the right-hand side of equation (29) will again become infinite for $x = 1$. The values of b (or n) for which the product on the right-hand side of (29) may remain finite can easily be found if we write it in the form $F(a, b, c, x) \div (1-x)^{-b}$ and observe that, whereas the hypergeometric series in the numerator can be written as

$$1 + \frac{ab}{1!c} x + \frac{a(a+1)b(b+1)}{2!c(c+1)} x^2 + \dots,$$

a binomial expansion of the denominator yields

$$1 + \frac{b}{1!} x + \frac{b(b+1)}{2!} x^2 + \dots$$

We see that both series become identical if (1) $b = 0$ (i.e., $n = 2$), (2) $a = c = 1/2$ (i.e., $n = -1$). Either case corresponds to the ratio of specific heats $\gamma = 4/3$ and the equilibrium of our configuration is clearly neutral. For any other value of γ —greater or smaller than $4/3$ —the amplitude of the radial oscillations becomes, however, infinite at $x = 1$, so that the boundary condition on the surface cannot be met. The conclusion follows, therefore, that a gaseous configuration of matter in hydrostatic equilibrium, obtained by a homologous transformation of a polytrope $n = 5$ into a sphere of finite dimensions, is incapable of exhibiting radial oscillations of finite frequency or amplitude.

It is a known fact that free periods of radial oscillations of polytropic gas spheres decrease, in general, with increasing degree of central condensation.⁷ Free periods of various modes of the polytrope $n = 0$ can, therefore, be regarded as upper bounds of the respective quantities for the polytropic family of models. On the other hand, when $n = 5$, no free periods were found to exist; their lower bound for the polytropic family of models therefore appears to be zero. It should be stressed, however, that the non-existence of finite periods of free oscillation of the polytrope $n = 5$ is *not* a consequence of the fact that the ratio of its central density to the mean density is infinite, but rather of the *rate* at which the density diminishes with increasing distance from the center. Sterne⁴ has, in fact, investigated another model, characterized by infinite central condensation, in which the density falls off as the inverse square of the distance from the center; and this model was found to admit of a whole spectrum of discrete periods of radial oscillations. On the other hand, the polytrope $n = 5$, for which the density in the envelope falls off approximately as inverse fifth power of the distance from the center, proved incapable of such oscillations. It appears therefore, that for spherically symmetrical configurations of compressible gas, the whole mass of which is confined to the center and is surrounded by an envelope which does not contribute appreciably to the gravitational potential within the body—a configuration rather loosely termed in the literature as the Roche model—a certain critical value of the density gradient in the envelope must exist beyond which radial oscillations are no longer possible. This critical value of $(r/\rho) (d\rho/dr)$ must, moreover, be between -2 and -5 . Sen⁸ claimed recently to have proved that, if the density in the envelope is assumed to vary as r^{-p} , where p is a positive integer, a failure of the convergence of a series expansion of the solution of the governing differential equation implies the non-existence of any permissible frequency of radial oscillation of the respective configuration if $p > 3$. Although the correctness of a part of Sen's investigation was challenged by Cowling,⁹ Sen's criterion appears to be consistent with the particular result established above for the polytrope $n = 5$. A generalization of Sen's work for non-integral values of p and a consequent clarifica-

tion of the limit of instability of the Roche model for radial oscillations are much to be desired.

¹ *M. N.*, **79**, 2 (1918); or *Internal Constitution of the Stars*, Cambridge, 1930, pp. 186-188. In his papers Eddington gave the explicit form of the equation (6) corresponding to the polytropic index $n = 3$ ("standard model"). The general form of equation (6) for unrestricted n was first explicitly stated by Miller, *M. N.*, **90**, 59 (1929).

² Cf., for instance, Bromwich, *Introduction to the Theory of Infinite Series*, Cambridge, 1908, p. 35.

³ In the notations of Courant-Hilbert, *Methoden der Mathematischen Physik*, Berlin, 1931, vol. I, p. 77.

⁴ *M. N.*, **97**, 582 (1937).

⁵ *Ibid.*, **90**, 59 (1929).

⁶ Cf., for instance, Chandrasekhar, *An Introduction to the Study of Stellar Structure*, Chicago, 1939, p. 97.

⁷ Cf. Miller, op. cit., the same fact holds true for non-radial oscillations as well (Cowling, *M. N.*, **101**, 367, 1942).

⁸ *Proc. Nat. Acad. Sci., India*, **A12**, 99 (1942).

⁹ *Math. Reviews*, **8**, 60 (1947).

EFFECTS OF DIFFERENT PROTEIN AND RIBOFLAVIN CONTENTS OF DIET UPON THE CHEMICAL COMPOSITION OF THE BODY*

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It has been shown that storage of riboflavin in the liver of the rat not only is correlated with the riboflavin intake¹ but also tends to increase with the protein content of the diet.²⁻⁶ Some experiments have also shown increased storage of protein, with or without decrease of fat, in the body at increased levels of nutritional intake of riboflavin.⁷⁻⁹ The present paper reports experiments upon the effects of varying both protein and riboflavin intakes upon the protein and riboflavin contents of the whole carcass as well as of the liver.

Thirty-day-old rats from the colony maintained in this laboratory were used as the experimental animals, and the period of experimental feeding was 30 days. The paired feeding method was used. The rats on the low protein diet were restricted to the amount of food eaten by those on the high protein, and those on high riboflavin were restricted to the amount eaten by those on the low riboflavin diet. Litter-mates matched as to sex were used on the diets being compared.

As seen in table 1, increasing the protein content from 12 to 32 per cent, in a diet containing 7 μ g. of riboflavin per gram of air-dry food, resulted in

TABLE 1
EFFECT OF INCREASING PROTEIN IN A DIET OF LIBERAL RIBOFLAVIN CONTENT: 7 μ g.
PER GRAM

(Average of 6 comparisons)

FINDINGS	WITH 12% PROTEIN DIET	WITH 32% PROTEIN DIET
Gain in body weight, g.	51.	68.
Liver weight, g.	5.94	7.88
Nitrogen in carcass, %	3.12	3.39
Nitrogen in liver, %	2.75	3.01
Riboflavin in carcass, μ g.	4.93	5.49
Riboflavin in liver, μ g./g.	28.1	31.9
Total nitrogen in body, g.	2.78	3.44
Total protein in body, g.	17.3	21.5
Total protein in body, %	16.95	18.42
Riboflavin per g. rat, μ g.	5.70	6.63
Total riboflavin in body, μ g.	631.	824.

increased growth and liver weight; and more growth per gram of food consumed, though the percentage utilization of protein was lower on the diet of higher protein content. On the higher protein diet the body retained more riboflavin, more protein, less fat and more water.

Table 2 shows the results of corresponding experiments with three diets containing uniformly 12 per cent protein but with 1, 3 and 9 μ g. of ribo-

TABLE 2
EFFECT OF INCREASING RIBOFLAVIN IN A 12% PROTEIN DIET

(Average of 5 Comparisons)

FINDINGS	WITH 1 μ g. OF RIBOFLAVIN/G.	WITH 3 μ g. OF RIBOFLAVIN/G.	WITH 9 μ g. OF RIBOFLAVIN/G.
Gain in body weight, g.	35.	38.	41.
Liver weight, g.	4.81	4.74	4.81
Nitrogen in carcass, %	2.91	2.92	2.95
Nitrogen in liver, %	2.84	2.85	2.87
Riboflavin in carcass, μ g./g.	5.19	5.53	6.56
Riboflavin in liver, μ g./g.	20.28	26.00	30.50
Total nitrogen in body, g.	2.23	2.26	2.71
Total protein in body, %	16.66	16.93	17.22
Riboflavin per g., μ g.	6.11	6.95	8.14
Total riboflavin in body, μ g.	468.	544.	632.

flavin per gram of air-dry food, respectively. Here the increase of riboflavin intake clearly increased the riboflavin content both of the carcass and of the liver, and both the amount of riboflavin per gram of body weight and

the total weight of riboflavin in the body; while the percentages of nitrogen were not significantly changed, either in the carcass or the liver, or in the body as a whole, by these differences of riboflavin intake at this 12 per cent level of food protein.

The same three levels of riboflavin intake were then tested with a different basal ration of moderately higher protein content—viz., 20 per cent of protein in the air-dry food mixture—with the results shown in table 3. Here the increases of intake of riboflavin had no significant influence upon the growth of the body or the amount of nitrogen it retained, either in carcass or liver; but definitely increased the riboflavin content of the liver and of the body as a whole. The percentage of total protein in the body as a

TABLE 3
EFFECT OF INCREASING RIBOFLAVIN IN A 20% PROTEIN DIET
(Average of 5 Comparisons)

FINDINGS	WITH 1 μ O. OF RIBOFLAVIN/G.	WITH 3 μ G. OF RIBOFLAVIN/G.	WITH 9 μ G. OF RIBOFLAVIN/G.
Gain in body weight, g.	40.	43.	41.
Liver weight, g.	5.45	4.79	4.82
Nitrogen in carcass, %	3.39	3.23	3.30
Nitrogen in liver, %	3.15	3.07	3.00
Riboflavin in carcass, μ g./g.	5.08	5.08	5.50
Riboflavin in liver, μ g./g.	17.85	24.38	29.06
Total nitrogen in body, g.	2.45	2.54	2.57
Total protein in body, %	18.00	18.20	18.60
Riboflavin per g., μ g.	6.04	6.67	7.17
Total riboflavin in body, μ g.	438.	511.	537.

whole showed a very slight trend toward increase at the successively higher levels of riboflavin intake, consistently with the well-known "concentration" or "mass action" principle and the protein-riboflavin relationship which is being established by current research; but the measured differences are too small to be statistically significant, so that the effect of the protein-riboflavin relationship upon protein retention in the body is practically plateaued at the intake levels of these experiments.

In all of the above-described experiments, the "paired-feeding" method was used. *Ad libitum* feeding was also used in some cases with the following results: In these experiments as in those above, increased nutritional intake of riboflavin, within the range 1 to 9 μ g. per gram of food, tended to increase the amounts stored in the body, both when the protein level was 12 and when it was 20 per cent of the air-dry food. The diet of highest riboflavin content was eaten in largest amount and resulted in largest gains of body weight and of riboflavin content of body; but the protein-riboflavin relationship was less clearly shown with *ad libitum* than with "paired" feeding.

Summary.—Paired-feeding experiments showed an increase of both riboflavin and protein content of liver and carcass on a diet containing 7 μ g. of riboflavin per gram when the protein content of the diet was increased from 12 to 32 per cent.

Increasing the riboflavin content of a 12 per cent protein diet from 1 to 9 μ g. per gram resulted in slight increase in weight, in fat and in total protein stored. The riboflavin in liver and carcass was also increased.

The same increase, from 1 to 9 μ g. of riboflavin per gram of air-dry food, on a 20 per cent protein diet had only slight influence upon growth or nitrogen stored, but increased the storage of riboflavin.

When food was allowed *ad libitum*, increasing the riboflavin in a 20 per cent protein diet from 1 to 9 μ g. per gram resulted in increased appetite, growth and retention of protein and riboflavin, though the percentages of protein were less because of the greater gain in body weight.

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ON THE FREQUENCY AND TRANSMITTED CHROMOSOME ALTERATIONS AND GENE MUTATIONS INDUCED BY ATOMIC BOMB RADIATIONS IN MAIZE*

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Samples of maize seed of the single cross L289 \times I205 were placed on some of the ships within the target area at Bikini at the time of the atomic

bomb test of July 1, 1946. At about the same time Dr. L. F. Randolph exposed duplicate samples to x-rays at Cornell University. Plantings were made at the California Institute of Technology of both series together with untreated controls. From these plants, sporocyte samples were collected by Dr. Randolph for studies at Cornell University on the visible chromosomal changes induced by the radiations (Randolph, Longley and Li *in press*). Studies on hereditary effects have been carried on at the California Institute of Technology in coöperation with the United States Department of Agriculture and the Naval Medical Research Section of Operation Crossroads.

Seedling comparisons supplemented later by pollen sector frequencies indicated one lot of seed exposed to atomic bomb radiations has received a dosage equivalent in biological effects to slightly below 15,000 r units of x-ray. Most of the studies have been made on this lot and on the comparable lot exposed to 15,000 r units x-ray.

In maize, chromosomal alterations can be detected readily by partial pollen sterility, unless the alteration involves only a very small portion of a chromosome. Examination of 751 plants of the unexposed controls showed only normal pollen.

Plants from irradiated seeds are mosaics of various sectors, each of which is assumed to be the progeny of a single meristematic cell of the multicellular growing tip of the embryo. Observations on the pollen indicated that about 90 per cent of the tassels of the Bikini lot had one or more sectors showing chromosome alterations.

Three plants were outcrossed to normals or to plants of low dosage when normal plants were not available. The outcross plants were derived from one normal gamete and one from the irradiated source, and should thus give a direct frequency of such transmissible effects as could be detected or recognized by the techniques employed.

In order to obtain a large number of chromosomal alterations for cytological study, ten seeds of each outcross were planted. The number of partial-sterile plants obtained was expected to be a little higher than random, since

1. Pollen was taken only from plants known to have at least one sector of partial sterility,
2. The majority of these pollinations were made on low dosage lots rather than on normal controls,
3. In the outcrosses of the Bikini or x-ray lots as female parents, seed was selected from poorly filled portions of the ear. Such parts are most likely to be partial-sterile sectors.

The observed frequencies were

	TOTAL PLANTS	PER CENT PARTIAL- STERILE
Normal or low dosage \times Bikini pollen	3176	4.7
Normal or low dosage \times 15,000 r x-ray	2582	6.0
Bikini \times normal pollen	5456	6.4
15,000 r x-ray \times normal pollen	3248	7.8

An unselected sample should give a somewhat lower frequency of transmitted partial-sterile plants, probably 4.5 or 5.0 per cent.

All partial-sterile plants were outcrossed to standard stocks to establish lines for cytogenetic study.

Some of the normal plants were self-pollinated to obtain mutant characters for other studies. In all, 1231 self-pollinated ears were harvested from the Bikini lot, and 655 from the corresponding 15,000 r x-ray lot. Of these 26 per cent of the ears from the Bikini lot and 23 per cent of those from x-ray were tabulated as segregating for endosperm characters such as white endosperm, sugary, brittle, opaque, shrunken, many types of defective endosperm and also several viviparous types. No doubt many defective types were overlooked.

Seedling tests are in progress and a test for mature plant characters will be made. Conspicuous seedling characters have been found to be even more frequent than the endosperm defects. The seedling characters include chlorophyll deficiencies such as white (albino), pale yellow and yellow lethals, pale green and yellow-green seedlings, virescents, piebald, zebra, striate and striped seedlings. Some appear to be mutable. A wide range of other characters such as glossies, dwarfs and a host of morphologically aberrant types have also appeared, many of which are unlike any described in the maize literature.

Because of the difficulty of establishing any objective standards for detecting and classifying miscellaneous mutations, no plans had been made for studies on mutation frequency and accordingly no controls were self-pollinated to test for the presence of recessive gene defects in the stock used. Some recessives could be carried in the commercial inbred lines, L289 and I205, used in making up the single cross, but not in such frequencies as were obtained. A number of control plants were used as pollen parents in a series of outcrosses with no clear evidence of any recessive having been carried in the control parent. Whenever the source could be determined the recessive came from the irradiated parent. It is possible that a minor portion of the gene defects may have been present in the stock, but at most it could have been only a small fraction. Additional tests will be made the coming summer including a study of the lower dosages and a checkup of the controls.

From data thus far obtained, it appears that most of the outcross plants carried one or more recessive mutations from the irradiated parent. Thus more than 50 per cent of the functioning gametes of plants from seed exposed to 15,000 r units x-ray or to the nearly equivalent atomic radiation exposure carry one or more gene mutations caused by the radiations.

The dormant seeds in these tests were exposed to much greater irradiation than the lethal dose for most animals or for actively growing plant tissue. But roughly similar frequencies obtained from 15,000 r x-ray and the nearly equivalent Bikini lot support the expectation that atomic bomb radiations have about the same heritable effects as comparable doses of x-rays.

Summary.—Maize seed receiving atomic bomb radiations equivalent to nearly 15,000 r units x-ray were tested for hereditary chromosomal alterations and gene mutations.

The frequency of chromosomal alterations as indicated by partial pollen sterility was 4.7–6.4 per cent in progenies which were not entirely random. It is estimated that a random frequency would be between 4.5 and 5.0 per cent.

The frequency of gene mutation was extremely high. Probably more than 50 per cent of the gametes of the exposed generation carried one or more gene mutations.

The frequencies of chromosomal alterations and gene mutations in a lot exposed to 15,000 r units x-ray were roughly equivalent to the frequencies obtained in the Bikini lot.

* Coöperative investigations of the Kerckhoff Laboratories of Biology, California Institute of Technology, and the Division of Cereal Crops and Diseases; Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture.

This is a brief report based on a coöperative program carried on at the California Institute of Technology by a group of investigators including Dr. A. E. Longley of the United States Department of Agriculture, Prof. E. F. Frolik of the University of Nebraska, Dr. E. E. Dale of Union College and the following graduate student assistants: C. H. Li, K. L. Retherford, Earl P. Patterson, Wayne F. Keim and Don Robertson.

THE RELATION BETWEEN NICOTINIC ACID AND CARBOHYDRATES IN A SERIES OF MAIZE ENDOSPERM GENOTYPES*

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It has been shown by Burkholder, McVeigh and Moyer,¹ by Barton-Wright,² and by Mather and Barton-Wright³ that kernels of surgary maize

are about twice as high in nicotinic acid as those of starchy maize. In the first-mentioned paper, kernels of a group of sugary strains were reported to average 34.6 micrograms of nicotinic acid per gram of tissue, as contrasted with 20.8 for starchy strains; in the last two papers the averages were 26.2 for sugary strains and 15.8 for starchy. The hereditary differences in the carbohydrates in all these strains were presumably controlled by the allelic genes Su_1 and su_1 , which act in such a way that kernels homozygous for the recessive su_1 are sugary, while those carrying one or more doses of Su_1 are starchy. No studies of the products of controlled crossing between Su_1 and su_1 strains were reported, but Mather and Barton-Wright³ analyzed starchy and sugary kernels from open pollination on ears of five sugary lines. These kernels, whose triploid endosperms were of the genotypes $Su_1su_1su_1$ and $su_1su_1su_1$, respectively, showed essentially the same difference in nicotinic acid content as that cited above. (Triploidy is normal in the maize endosperm, owing to fusion of two female polar nuclei with one male gamete.)

Cameron⁴ studied the effects of two pairs of alleles, su_1^{am} su and Du du , on the reserve carbohydrates in a series of maize endosperm genotypes. The gene su_1^{am} is a member of the Su_1su_1 series³ (for convenience, subscript numerals for this allelic series are omitted hereafter); whereas Du and du are wild type and recessive forms at an independent locus.⁵ These genes interact so that endosperms carrying one or more doses of both su^{am} and Du are primarily starchy type (opaque and smooth) in appearance, while those homozygous for su or du , or both, are sugary type (translucent and wrinkled). Chemical analyses, which provide a more sensitive measure of the genetic effects, showed that the homozygous double-recessive genotype $su\ su\ su\ du\ du\ du$ was the lowest in starch and the highest in soluble carbohydrates and that starch increased and soluble carbohydrates decreased in a rather definite pattern with successive doses of either su^{am} or Du . First doses were usually the most effective, su^{am} was more effective than Du , and one dose of each gene was usually more effective than three doses of either alone. The behavior of the entire series of genotypes with respect to starch may be seen in table 1. Notations in the dosage column of this table make clear the identity of each genotype in terms of its gene dosage: 1-0 for example, carries one dose of su^{am} and none of Du , and therefore two doses of su and three of du .

The present paper reports data on the nicotinic acid content of this genotypic series, together with exploratory data on the thiamin and biotin content of selected genotypes.

Methods.—Mature maize endosperms from the same or sister ears of the series which had served for the carbohydrate studies⁴ were used for the present analyses. The ears were produced in a single plot in one season,

TABLE 1
NICOTINIC ACID AND STARCH IN MATURE MAIZE ENDOSPERM GENOTYPES RESULTING FROM COMBINATIONS OF THE TWO GENE PAIRS $su^{am} su$
AND $Du du$

GENERAL PHENOTYPIC CLASS	GENOTYPE	GENE DOSAGE, <i>su^{am}Du</i>	PER CENT STARCH* (DRY- WEIGHT BASIS)	MICROGRAMS NICOTINIC ACID PER GRAM OF TISSUE (AIR-DRIED)				
				INDIVIDUAL GENOTYPE	SUBGROUP MEAN	GROUP MEAN		
Primarily starchy (opaque and smooth)	<i>su^{am}su^{am}su^{am}</i>	3-3	80.7	21.7	22.2	24.2		
	<i>su^{am}su^{am}su^{am}</i>	3-2	77.7	22.3				
	<i>su^{am}su^{am}su</i>	2-3	76.0	21.4				
	<i>su^{am}su^{am}su</i>	2-2	79.6	23.5	25.8			
	<i>su^{am}su^{am}su^{am}</i>	3-1	72.7	22.4				
	<i>su^{am}su^{am}su</i>	2-1	71.8	29.4				
	<i>su^{am}su</i>	1-3	69.7	22.2	47.4			
	<i>su^{am}su</i>	1-2	70.3	25.5				
	<i>su^{am}su</i>	1-1	58.6	29.4				
	Sugary (translucent and wrinkled)	<i>su^{am}su^{am}su^{am}</i>	3-0	51.5	44.3		51.6	50.5
		<i>su^{am}su^{am}su</i>	2-0	42.0	46.2			
		<i>su^{am}su</i>	1-0	33.6	51.6			
<i>DuDuDu</i>		0-3	32.2	56.3	56.7			
<i>DuDuDu</i>		0-2	30.0	48.2				
<i>DuDuDu</i>		0-1	27.2	50.4				
<i>DuDuDu</i>		0-0	19.2	56.7	51.6			
<i>DuDuDu</i>								

* Data from Cameron.⁴

with the exception of the genotype *su^{am} su su du du du*, which was grown in a different year.

Vitamin values are reported on an air-dry basis. Since earlier measurements had shown the entire range in moisture content to be from 7.6 to 10.5%, little error in relative values is introduced. Endosperms from each of two ears of each genotype were pooled and ground to 60 mesh in a Wiley electric mill, and the resulting material was thoroughly mixed. For the nicotinic acid assays reported in table 1, 1.0 g. of each sample was hydrolyzed by autoclaving with 50 cc. of 1 *N* sulfuric acid for 30 minutes at 15 lbs. pressure, and the suspensions were neutralized, filtered and made up to volume. The growth of *Lactobacillus arabinosus*, as determined by acid production during 72 hours at 35°C., was taken as a measure of the nicotinic acid. The culture medium and the general procedure were those described by The Association of Vitamin Chemists, Inc.⁶ Calculations of nicotinic acid were made from the averages of three or more assays (each in duplicate) which agreed within 10% of the total average of duplicates representing six concentrations of added endosperm hydrolyzate. A sample of enriched wheat flour obtained from The Association of Vitamin Chemists, Inc., was assayed as a control in two experiments. The values obtained were within 3% of the range already reported by the Association (in personal communication to the junior author) for this material.

For the thiamin and biotin determinations certain strains of *Neurospora* were used, their response being measured by the dry weights of mycelial pads formed in a 72-hour growth period in liquid medium. The general techniques of culturing *Neurospora* strains, the means of supplying supplement and other procedures have been described.⁷ Strain 18558, a thiamineless mutant which requires vitamin thiazole or intact thiamin,⁸ was used for the thiamin estimations. The biotin estimation was carried out with wild type strain 14, which is deficient in the ability to synthesize biotin. The maize hydrolyzates for the biotin assay were the same as those used for nicotinic acid determinations. The samples for thiamin assay were prepared by simultaneous takadiastase and papain treatment in acetate buffer, according to the method of Cheldelin, *et al.*⁹ For each determination a standard curve was run in duplicate, and five levels of endosperm hydrolyzate were used. The average of all values read from the relatively straight-line portion of the curve was taken in calculating the vitamin levels.

Results.—In table 1 the nicotinic acid content of the genotypes is shown in relation to the previously determined values for starch.⁴ Whereas the starch content had shown a stepwise response to gene dosage, ranging from 19.2% starch in genotype 0-0 to 80.7% in genotype 3-3, the content of nicotinic acid appears to be less sensitive. It is relatively low (mean, 24.2 micrograms per gram of tissue) in the group of nine starchy types that

carried both *su^{am}* and *Du*, and higher by a factor of approximately 2 (mean, 50.5 micrograms per gram) in the group of seven types that lacked one or both of these alleles. The difference between the two means is highly significant, with a *P* value of less than 0.01. Within each of these two groups the variation among individual values is non-significant by the X^2 test; nevertheless, there is a marked tendency for nicotinic acid to reflect changes in genotype correlated with changes in starch content. This has been indicated in a column of subgroup means. Thus genotype 0-0 had a nicotinic acid content of 56.7 micrograms per gram of tissue; genotypes 0-1, 0-2 and 0-3 averaged 51.6 micrograms per gram; genotypes 1-0, 2-0 and 3-0 averaged 47.4, and so on. For the entire series, the correlation coefficient between starch and nicotinic acid is -0.97 , with a *P* value of less than 0.01.

It has been shown that the apparent nicotinic acid content of cereal products is influenced by the method of extraction; thus wheat autoclaved with 0.1 *N* sulfuric acid assayed 37 micrograms of nicotinic acid per gram of

TABLE 2
EFFECT OF STRONG AND MILD HYDROLYSES ON THE APPARENT NICOTINIC ACID CONTENT OF FIVE MAIZE ENDOSPERM GENOTYPES

GENE DOSAGE, <i>su^{am}Du</i>	NICOTINIC ACID RELEASED BY		
	STRONG HYDROLYSIS, MICROGRAMS PER GRAM OF TISSUE	MILD HYDROLYSIS MICROGRAMS PER GRAM OF TISSUE	PER CENT OF STRONG HYDROLYSIS
3-3	21.7	15.0	69.1
1-1	29.4	19.3	65.6
3-0	44.3	29.6	66.8
0-3	56.3	28.3	50.3
0-0	56.7	39.0	68.8

tissue, but when treated with 1.5% sodium hydroxide or 3 *N* hydrochloric acid it assayed 72 to 75 micrograms per gram.¹⁰ One *N* sulfuric acid also gives maximum nicotinic acid values in cereals,¹¹ and the data reported in table 1 were obtained by its use. To determine whether there might be differences among the genotypes in the proportion of nicotinic acid released by strong and mild hydrolyses, five of the genotypes were assayed after autoclaving with 0.1 *N* sulfuric acid. The results of these assays are presented in table 2 and compared with those from table 1. In each case the value obtained by mild hydrolysis was markedly lower than that obtained by strong hydrolysis. With the exception of genotype 0-3, the percentage of total nicotinic acid made available by this mild treatment was about the same for all, however, and this suggests that *su* and *du* exert no differential action in this respect. Without further tests it cannot be determined whether the one notably lower percentage (50.3, for genotype 0-3) has significance.

Results of exploratory assays for thiamin and biotin, carried out on the same five genotypes, are shown in table 3. These data must be considered only approximate, but they indicate that the two vitamins bear a relationship to the carbohydrate system similar to that of nicotinic acid, in that they are low in starchy types and higher in sugary ones. The biotin level found for genotype 3-3 agrees well with the value reported by Cheldelin and Williams¹² for white corn meal, but the thiamin level for this genotype is less than one-tenth as much as that reported by Van Lanen, Tanner and Pfeiffer¹³ for a composite sample of yellow maize.

Discussion.—The data of Burkholder, McVeigh and Moyer,¹ together with those of Mather and Barton-Wright³ and of the present paper, demonstrate a general correlation between sugary-type maize endosperm and relatively high nicotinic acid content. Three different gene combinations, *su^{am} du*, *su Du* and *su du*, when homozygous for their recessive members, are sugary type and high in nicotinic acid, while two combinations, *Su Du* and *su^{am} Du*, are starchy type and low in nicotinic acid. Thus

TABLE 3
THIAMIN AND BIOTIN CONTENT OF FIVE MAIZE ENDOSPERM GENOTYPES

GENE DOSEAGE, <i>su^{am} Du</i>	MICROGRAMS PER GRAM OF TISSUE	
	THIAMIN	BIOTIN
3-3	0.18	0.07
1-1	0.37	0.09
3-0	0.42	0.14
0-3	0.98	0.13
0-0	0.88	0.15

changes involving either the *Su su^{am} su* series or the *Du du* pair are independently effective. Endosperm tissue from the gene combination *Su du* has not been assayed, but since this is a starchy type it is probable that the nicotinic acid content would be low.

The present data, like those on the carbohydrates,⁴ have been presented on a percentage basis, or its equivalent. In this genotypic series, however, about 1.37 sugary-type endosperms were required to equal the weight of 1 starchy-type. If the number of cells laid down in an endosperm were essentially the same in the two types (with the weight difference due primarily to differential amounts of stored carbohydrates), and if the production of the vitamins were a cell function unrelated to the genes in question, then sugary tissue might be expected to yield about 1.37 times as much as an equivalent weight of starchy tissue. Such a situation, however, does not account for the extent of the differences found. Studies on starchy maize hybrids¹⁴ have shown that variation in nicotinic acid content can also occur as a function of the hybrid, and of the year and the location of the planting. In the present series these factors were not variables.

For comparable types, the nicotinic acid values reported here average higher than those of Mather and Barton-Wright;³ they agree rather well with the starchy-type average of Burkholder, *et al.*,¹ but are higher than the sugary-type average reported by these workers. These differences are probably a function of the genetic backgrounds involved, for all these authors reported wide variation in nicotinic acid for both sugary and starchy kernels of various varieties. Their assays differed also in that they were apparently made upon whole kernels.

The mechanisms underlying the relationships between the carbohydrates and the three B vitamins are not known, and the possibilities are many. The primary action of the genes may relate either directly or indirectly to the carbohydrates or to any one or all of the vitamins.

It has been suggested⁴ that in the starch-synthesizing processes certain steps relating to the action of phosphorylase are affected by *su* and *du*. If this is correct, changes in vitamin content may be secondary. This is plausible, since (a) vitamin content appears to be less sensitive than carbohydrate content to changes in genotype; and (b) the three vitamins all change in the same direction, as if in response to some general influence. The degree of starch accumulation might constitute such an influence. In starchy endosperms the cells become primarily storehouses for starch grains, and the production of other substances may be limited as a result.

In microorganisms shunt mechanisms exist,¹⁵ which, although often entirely inoperative in the presence of low concentrations of (soluble) carbohydrate, form substantial quantities of certain metabolic products when carbohydrate is plentiful. In the present case the sugary maize types, in which soluble carbohydrates accumulate, may produce greater quantities of vitamins by such a mechanism. It is also possible that a portion of the vitamins or some of their components are used up (from an otherwise constant store) in the process of polysaccharide synthesis, so that smaller amounts remain in starchy kernels than in sugary ones. Thus nicotinamide, which is related to cozymase, may suffer loss due to "attrition" or other causes¹⁶ in the course of this activity.

If it is the vitamins which the genes more directly control, different explanations are required. Conceivably, the greater quantities of vitamins in sugary genotypes could interfere with synthesis of starch or contribute to its breakdown. Again, in microorganisms it has been shown¹⁷ that high vitamin levels can cause metabolic upsets.

It has been pointed out (by E. G. Anderson, in personal communication to the writers) that thiamin and perhaps precursors of other vitamins may appear in the endosperm by translocation from the leaves. This could imply control of these substances by genes in the diploid plant tissues, which would require a reconsideration of the gene dosages involved.

Data are accumulating which make possible the breeding of maize for

greater nutritive value. It has been known for some time that the content of vitamin A-active carotenoids is controlled by a gene pair, Y_1 and y_1 ; yellow maize contains these carotenoids in direct linear proportion to the doses of Y_1 present; white maize lacks them.¹⁸ In studies on the inheritance of oil, inbreds of high and low oil content have been obtained, and genetic effects are detectable in their hybrids;¹⁹ the inheritance of proteins and amino acids is likewise being studied. In sweet corn, genes such as *du*, in conjunction with the common *su*₁, provide possibilities for the development of improved eating quality. The low nicotinic acid content of starchy maize is well known, especially because of its apparent relation to pellagra. A somewhat higher content can be obtained by selection among starchy hybrids,¹⁴ and for purposes for which sugary types can be utilized the still higher levels represent an additional advantage.

Summary.—In a series of relatively isogenic mature maize endosperm genotypes, inversely related changes in nicotinic acid and starch content were found to be conditioned by genes at both the *su*₁ locus and the *du* locus. Nicotinic acid content appeared somewhat less sensitive to changes in genotype than did starch content. On a percentage basis sugary types averaged about twice as high in nicotinic acid as starchy types.

The proportion of total nicotinic acid made available by mild hydrolysis was similar in all but one of a group of contrasting genotypes tested.

Exploratory assays for thiamin and biotin indicated that their content in the endosperm, like that of nicotinic acid, is low in starchy types and higher in sugary ones.

Possible mechanisms underlying these relationships are discussed.

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A GENE-CONTROLLED REACTION IN *NEUROSPORA* INVOLVING THE SYNTHESIS OF PANTOTHENIC ACID

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The postulate that the gene is the ultimate agent controlling biochemical reactions is accepted by many geneticists as a working hypothesis. The evidence is based on the fact that gene changes bring about changes in biochemical reactions, and sometimes create a "genetic block" which makes it impossible for products to be formed. Furthermore, each gene is assumed to be specific for a specific type of biochemical reaction, with the corollary

logically following that there exists a one-to-one relationship between the gene and the enzyme.^{1,2} The experimental evidence confirming this relationship is scanty, because its direct investigation is made difficult by inadequate information on known gene-controlled reactions, and the technical difficulties of carrying out many of these reactions *in vitro*.

That the ability to produce a specific enzyme is, in fact, inherited has been demonstrated in at least two cases. The clover, *Trifolium repens* has been shown to possess a genetically determined enzyme, linamerase, which hydrolyzes the glucosides, linamerin and lotaustralin.³ Some rabbits possess an atropinesterase in their blood which hydrolyzes atropine and monoacetylmorphine, and the ability to produce this enzyme is incompletely dominant to the condition in which the enzyme is absent.⁴ In both of these cases the enzyme activity can be demonstrated *in vitro*. The substrate and end-products have been chemically characterized, and the presence or absence of the enzyme shown to be an inherited trait.

It is the purpose of the present investigation to make a direct study of the gene-enzyme relationship in the fungus, *Neurospora*. Two strains of *Neurospora* obtained from Dr. G. W. Beadle at the California Institute of Technology were used, the Emerson Wild Type 5265A and Pantothenicless 5531A. The pantothenicless mutant is unable to grow unless supplied with pantothenic acid, a vitamin not required by the wild type in its medium. It has been reported that 5531 has the ability to synthesize both β -alanine and pantoil lactone (*DL*- α -hydroxy- β , β -dimethyl- γ -butyrolactone), and therefore presumably has a genetic block for the reaction coupling these fragments to produce pantothenic acid.⁵ This mutant differs from the wild type by a single gene, and the gene locus has been approximately located on one of the seven chromosomes of *Neurospora*.⁶

Experimental.—Both wild type and pantothenicless *Neurospora* were grown in basal medium⁷ with 1 γ per ml. of calcium pantothenate in the pantothenicless cultures. Mycelial pads were harvested at the end of 72 hours, washed thoroughly with sterile, distilled water and transferred aseptically to sterile flasks containing 25 ml. of distilled water. To one set of flasks 5 ml. each of 0.1 *M* solutions of β -alanine and pantoil lactone was added. The cultures were incubated at 28°C. with constant shaking. Flasks removed after different intervals of time were steamed for about ten minutes; the mycelium was harvested and dried at 90°C., and then weighed. The fluid contents of the flasks were assayed for pantothenic acid with *Lactobacillus arabinosus* according to the method of Skeggs and Wright.⁸ One per cent glucose (instead of two per cent) was used in the assay medium and growth response was measured turbidimetrically after an incubation period of 17 to 18 hours instead of acidimetrically after 72 hours' incubation. The amount of pantothenic acid produced in the medium per mg. dry weight of mycelium was calculated. The data from a 41-

hour run are presented in table 1. They show that the wild type produces considerable amounts of pantothenate in the presence of β -alanine and pantoyl lactone, and very little in their absence, whereas the pantothenic-less mutant produces none in either case. There can be little doubt that wild type *Neurospora* is capable of coupling the pantoyl lactone and β -alanine to form pantothenic acid, and that the pantothenicless mutant has a genetic block at this coupling reaction.

It should be noted, in connection with the experiment reported above, that no attempt was made to control the pH, but that the pH remained at about 6.0 during the course of the incubation. It was also found that toluene, which was used as a preservative in one experiment, completely inhibited the production of pantothenate by wild type.

In vitro experiments were carried out with a preparation made by growing large amounts of mycelium in minimal medium. Growth was per-

TABLE 1
PRODUCTION OF PANTOTHENATE BY INTACT, RESTING MYCELIUM OF WILD TYPE AND PANTOTHENICLESS *Neurospora* ("PRECURSORS" REFERS TO β -ALANINE AND PANTOYL LACTONE)

PERIOD OF INCUBATION (HOURS)	MICROGRAMS PANTOTHENATE PRODUCED PER MG. DRY WEIGHT MYCELIUM			
	WILD TYPE		PANTOTHENICLESS	
	PERCURSORS	NO PERCURSORS	PERCURSORS	NO PERCURSORS
0	0.01	0.01	0.01	0.00
5.5	0.09	0.01	0.01	0.01
16.5	0.35	0.04	0.01	0.03
22.5	1.23	0.11	0.03	0.03
28.5	2.50	0.11	0.02	0.03
41.0	5.00	0.06	0.02	0.02

mitted to proceed for 48 hours with aeration, and the mycelium then washed with distilled water and four successive portions of dry acetone. After being dried thoroughly the preparation was ground to a fine powder and stored in the refrigerator. The yield is about 20 to 23 g. of acetone-dried mycelium per 15 liters of culture fluid.

The effect of hydrogen-ion concentration on the production of pantothenate was tested by using 50 mg. of the dried mycelial preparation per flask suspended in 25 ml. of 0.04 *M* potassium phosphate buffer solution containing 1 ml. each of 0.1 *M* solutions of pantoyl lactone and β -alanine. The solutions were sterilized and the mycelial preparation added aseptically. The reaction mixtures were incubated at 25°C. and shaken constantly for 24 hours. They were then steamed, the solids separated by centrifugation and the centrifugates assayed for pantothenate. Since in alkaline solution there is a certain amount of synthesis of pantothenic acid from β -alanine and pantoyl lactone when these compounds are heated together in solution, a control series of flasks was set up which contained no mycelium. The contents of these flasks were assayed and the values obtained subtracted

from the pantothenate values found in the flasks with mycelium. The results expressed in micrograms pantothenate per mg. acetone-dried preparation are given in table 2. They show that the optimal pH range under the conditions of this experiment lies above pH 6.0.

TABLE 2

THE EFFECT OF HYDROGEN-ION CONCENTRATION ON THE SYNTHESIS OF PANTOTHENATE BY THE ACETONE-DRIED MYCELIUM OF WILD TYPE *Neurospora*

pH	MICROGRAMS PANTOTHENATE PRODUCED PER MG. MYCELIUM
5.0	0.01
5.5	0.29
6.0	0.70
6.5	0.79
7.0	0.59
7.5	0.62
8.0	0.59
9.0	0.54

The effect of omitting the precursors, β -alanine and pantooyl lactone, is shown in table 3. In this experiment incubation time was 24 hours at 25°C. The blank contained only phosphate buffer at pH 6.0 and the mycelium. The data demonstrate the dependence of the reaction on the presence of both of the precursors.

TABLE 3

THE EFFECT OF THE PRESENCE OF β -ALANINE AND PANTOYL LACTONE ON THE PRODUCTION OF PANTOTHENATE BY WILD TYPE *Neurospora*

	MICROGRAMS PANTOTHENATE PRODUCED PER MG. MYCELIUM
Pantooyl lactone + β -alanine	0.58
Pantooyl lactone	0.13
β -alanine	0.11
Blank	0.10

Table 4 shows the effect of increasing the concentration of the mycelial preparation on the amount of pantothenate synthesized during an incubation period of 24 hours at 25°C. The total amount of pantothenate produced per flask is given in the table.

The release of pantothenic acid preformed in the wild type mycelium used in the preceding experiments might be expected to have some effect on the results. For this reason intact and acetone-treated mycelia were assayed for their content of the vitamin. Preparations from fresh cultures were homogenized in acetate buffer at pH 4.5 and treated with Clarase and Caroid enzyme mixtures for 24 hours at 37°C.⁹ No pantothenate was detected by assay in the case of the intact mycelium, probably because of incomplete digestion. However, the acetone-treated mycelium contained 0.014 γ pantothenate per mg., a concentration very close to the pantothen-

ate content reported for *Oidium lactis*.¹⁰ If we accept this value as a reasonably accurate one for wild type *Neurospora* then it becomes quite evident that pantothenate must actually have been synthesized, and not merely released after having been formed during the actively growing phase of the mycelium.

Discussion.—The data presented demonstrate that there is an enzyme system present in wild type *Neurospora* which catalyzes the synthesis of pantothenic acid from β -alanine and pantoyl lactone. This reaction can be demonstrated both *in vivo* and *in vitro*, although the rate is not as great in the latter case. Efforts made to increase the activity of the acetone-dried preparation to at least that of the intact mycelium have not met with consistent results. At present it appears that glucose and coenzymes such as adenosine triphosphate, pyridoxal phosphate and Coenzyme I may stimulate the synthesis of pantothenate *in vitro*, but the proper conditions have not yet been worked out.

TABLE 4
THE EFFECT OF CONCENTRATION OF THE MYCELIAL PREPARATION ON THE SYNTHESIS OF PANTOTHENATE

MG. MYCELIAL PER FLASK	MICROGRAMS PANTOTHENATE PRODUCED PER FLASK
25	37
50	55
75	93
100	175

The present investigation indicates that the reaction leading to the production of pantothenate from β -alanine and pantoyl lactone in *Neurospora* should be useful in studying directly the gene-enzyme relationship in this organism.

The authors gratefully acknowledge the interest shown in their work by Dr. H. K. Mitchell of the California Institute of Technology whose suggestion concerning the possible usefulness of the pantothenicless mutant of *Neurospora* provided the initial stimulus for this investigation.

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MULTIPLICATIVE SYSTEMS, I

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1. *Introduction.*—The purpose of this note is to introduce a formalism suitable for the description of systems which consist of particles of different types, each particle transmuting with given probability into a group of particles of these types, the number of particles in the system varying with time.^{1, 2, 3} The type of a particle may refer to characteristics like position or momentum as well as to intrinsic properties.

The phase space in which we are interested here is that of all possible futures or genealogies of such systems, each such genealogy being considered as a point of the space. Given the elementary probabilities of transmutation, one can introduce a notion of probability or measure for certain subsets of the phase space.

The formalism is general enough to include as special cases the multiplication of bacteria, radioactive decay, cosmic ray showers, diffusion theory and the theory of trajectories in mechanical systems.

Detailed discussion of specific cases, at present in the form of reports, will be published elsewhere.

2. *Remark on Measure.*—It is convenient to have at hand some simple axioms on which the classical measure theory may be shown to rest. Suppose $\Gamma = \{\gamma\}$ is a point set, $I = \{i\}$ a class of distinguished subsets i of Γ called intervals, including the empty set \emptyset and the entire set Γ . Denote by J the class of all subsets $S = \Sigma i$ which are sums of a finite or countable class of intervals (all sums hereafter are supposed to be over a finite or countable number of summands). Suppose that intervals satisfy the axioms:

I.1. Every set S of J can be represented as a sum of mutually disjoint intervals.

I.2. The complement $i' = \Gamma - i$ of an interval i is in J .

I.3. The set product ij of two intervals i, j is an interval.

Assume further

M.1. To every interval i is assigned a non-negative real number $m(i)$ called its measure.

M.2. $m(\Gamma) = 1$.

M.3. If $i = \Sigma i_\mu$, where the i, i_μ are intervals and the i_μ are pairwise disjoint, then $m(i) = \Sigma m(i_\mu)$.

An additive class C of subsets of Γ is one such that

C.1. All intervals belong to C .

C.2. If A_1, A_2, \dots are in C , so is ΣA_μ .

C.3. If A is in C , so is A' .

The intersection of all additive classes consists of the Borel sets. Thus all Borel sets are contained in any additive class.

If one defines outer measure $O(U) = \text{glb}\{m(S); U \subset S \in J\}$ and inner measure $I(U) = 1 - O(U')$ as usual for an arbitrary subset U of Γ , one can show on the basis of the stated axioms that the class of all measurable sets M (for which $I(M) = O(M)$) is an additive class. Moreover, defining measure $m(M)$ for measurable sets to be the common value $I(M) = O(M)$, one can show $m(M) \geq 0$; $m(\Sigma M) = \Sigma m(M)$, for disjoint measurable sets M ; and if M is an interval, the assigned measure coincides with the original interval measure.

3. *The set Γ_t of Graphs.*—Consider t types of particles such that a particle of type i may produce, upon transformation, $j_1 + \dots + j_t \geq 0$ of such particles, of which j_ν are of type ν , $\nu = 1, \dots, t$. We suppose transformation times are the same for each type and hence that generations may be counted unambiguously. We agree to consider zero generation as consisting of one particle of a fixed type i . Then we consider the set Γ_t of all possible infinite histories or genealogies of such a particle, that is, the infinite records of the transformations of this particle and all its progeny through all generations $k = 0, 1, 2, \dots$

We may represent a genealogy in the plane if we make the following conventions:

(a) t different "colors" are assigned to the t types, a particle of type ν in the k th generation being represented by a dot of appropriate color.

(b) If a particle of type μ in generation k is transformed into no particle, i.e., if it dies or escapes, this is indicated by a sequence of Δ 's (Δ = death) vertically below it, one in each succeeding generation.

(c) If a particle of type μ in generation k is transformed into $j_1 + \dots + j_t > 0$ particles, j_ν of type ν , this is indicated by a branching from the corresponding dot of color μ in the k th generation to $j_1 + \dots + j_t$ dots, j_ν of color ν , in the $k + 1$ st generation, dots of the same color being placed consecutively and colors ranging in order $\nu = 1, \dots, t$ from left to right.

Thus we may regard the set Γ_t of all such graphs γ in the plane. If γ is a graph, γ_n denotes the upper segment of γ from generation 0 through generation n . If $\gamma = \tilde{\gamma}$, then $\gamma_n = \tilde{\gamma}_n$ for all n . If $\gamma \neq \tilde{\gamma}$, define $k(\gamma, \tilde{\gamma})$ as the least integer n for which $\gamma_n \neq \tilde{\gamma}_n$.

The set Γ_t has the natural metric: $d(\gamma, \tilde{\gamma}) = 0$ for $\gamma = \tilde{\gamma}$, $d(\gamma, \tilde{\gamma}) = 1/k(\gamma, \tilde{\gamma})$ for $\gamma \neq \tilde{\gamma}$. The metric space Γ_t resulting is 0-dimensional, separable, and complete, but *not* locally compact.

A graph γ is said to terminate in case it contains no particles (only Δ 's) in some generation. The set T of all terminating graphs may be split into the disjoint summands T_0, T_1, \dots , where T_n denotes the set of all

terminating graphs $\tau^{(n)}$ which contain at least one particle in generation n , but none in the next.

By an interval of order n is meant the set $i(\tau^{(n)})$ of all graphs γ such that $\gamma_n = \tau_n^{(n)}$ where $\tau^{(n)}$ is a particular graph in T_n . The only interval of order 0 is Γ , itself. We now define *interval* to be either θ , or any single graph γ , terminating or not, or any interval of order n , $n = 0, 1, \dots$. It is easy to see that axioms I.1, 2, 3 are satisfied. Indeed we have the simple property that the intersection ij of two intervals is either θ or i or j .

4. *Measure in the Space of Graphs.*—While the notions of graph, distance and interval are geometric in character and depend only on the number t of types, measure may be introduced in various ways. One of the simplest, but by no means the only one to which the theory has been applied, may be imposed as follows. Suppose we assign a probability $p(i; j_1, \dots, j_t)$ to the event that a particle of type i should produce, upon transformation, $j_1 + \dots + j_t$ particles, j_r of type r . Then every segment γ_n of a graph γ has an associated probability $p(\gamma_n)$ that the event described by γ_n should occur. If we assign to intervals a measure by $m(\theta) = 0$, $m(\tau^{(n)}) = p(\tau_{n+1}^{(n)})$, $m(i(\tau^{(n)})) = p(\tau_n^{(n)})$, and $m(\gamma) = \lim p(\gamma_n)$ for γ non-terminating it turns out that M.1, 2, 3 are satisfied and thus Borel sets based on our intervals are measurable in the sense of §2. (Non-compactness of intervals makes M.3 non-trivial.)

The procedure applies in much more general systems where the transition probabilities are functions of the time.

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ON THE ZEROS OF THE DERIVATIVE OF AN ENTIRE FUNCTION OF FINITE GENRE

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Let $E(z)$ denote an entire function of genre p :

$$E(z) = e^{P(z)} z^q \prod_{j=1}^{\infty} [1 - (z/z_j)] \exp \sum_{k=1}^p [(z/z_j)^k/k],$$

where $P(z)$ is a polynomial of degree p_1 and $p = \max(p_1, q)$. Clearly, the zeros of its derivative $E'(z)$ depend not only upon the zeros z_j of $E(z)$ but

also upon the polynomial $P(z)$. This contrasts with the fact that the zeros of the derivative of a polynomial depend only upon the zeros of the polynomial. It accounts for the difficulty experienced by some previous investigators who, seeking to extend to entire functions the well-known results on the zeros of the derivative of a polynomial, found it necessary to assume $P(z) \equiv 0$ or to restrict p to the values 0 or 1.¹

In the present note, the difficulty due to the $P(z)$ is circumvented by establishing for $E'(z)$ the following new representation.

THEOREM 1. *Let $E(z)$ be an entire function of genre p . Let $z_0 = 0$, z_1, z_2, \dots denote the zeros of $E(z)$ with the multiplicities m_0, m_1, m_2, \dots , respectively, and let Z_1, Z_2, \dots, Z_p denote any p zeros of $E'(z)$. Then*

$$E'(z) = E(z) \sum_{j=0}^{\infty} \{m_j/(z - z_j)\} \Pi_{k=1}^p [(Z_k - z)/(Z_k - z_j)].$$

Theorem 1 may be proved by extending to meromorphic functions our recent results² on the zeros of rational functions of the form

$$F(z) = \sum_{j=0}^{p-1} A_j z^j + \sum_{j=1}^n m_j/(z - z_j),$$

where the A_j are arbitrary complex constants and the m_j are points in a convex sector. It may also be proved directly by eliminating $P(z)$ from $E'(z)$ by use of the equations $E'(Z_k) = 0$, $k = 1, 2, \dots, p$.

An immediate consequence of Theorem 1 is

THEOREM 2. *If Z_1, Z_2, \dots, Z_{p+1} are any $p + 1$ zeros of the function $E'(z)$, then*

$$\sum_{j=0}^{\infty} m_j / [(Z_1 - z_j)(Z_2 - z_j) \dots (Z_{p+1} - z_j)] = 0. \quad (1)$$

The study of the argument of each term in equation (1) leads now to

THEOREM 3. *Let K denote the smallest convex infinite region which encloses all the zeros of $E(z)$ and let $S(K, \psi)$ denote the star-shaped region comprised of all points from which K subtends an angle of at least $\psi = \pi/(p + 1)$. Then at most p zeros of $E'(z)$ lie outside of $S(K, \psi)$.*

When $p = 0$, the region $S(K, \psi)$ coincides with K . Thus Theorem 3 is seen to be a generalization of the Lucas Theorem that any convex region enclosing all the zeros of a polynomial also encloses all the zeros of its derivative.

In particular, if K is chosen as a convex sector, the following result is obtained.

THEOREM 4. *Let α and β be non-negative numbers such that $\alpha \leq \beta$, $\alpha + \beta = \pi/(p + 1)$. If all the zeros of $E(z)$ lie in the sector $|\arg z| \leq \alpha$, then at most p zeros of $E'(z)$ lie in the sector $|\arg(-z)| \leq \beta$.*

Equation (1) also leads to an elementary proof of the following result of Laguerre and Borel.³

THEOREM 5. *Let $E(z)$ be a real entire function with only real zeros. Then*

$E'(z)/E(z)$ has at most p real and non-real zeros in excess of a single zero between each pair of successive zeros of $E(z)$.

All of the above theorems remain valid if $E'(z)$ is replaced by $H(z) = E'(z) + Q(z)E(z)$ where $Q(z)$ is an arbitrary (real, in the case of Theorem 5) polynomial of degree not exceeding $p - 1$. For, the function $E_1(z) = E(z) \exp. \int Q(z)dz$ is an entire function of genre p possessing the same zeros as $E(z)$ and its derivative $E_1'(z) = H(z) \exp. \int Q(z)dz$.

A more detailed account of the above results, together with additional applications, will be published elsewhere at a later date.

¹ Cf. the report on the zeros of entire functions by E. B. Van Vleck, "On the Location of the Roots of Polynomials and Entire Functions," *Bull. Amer. Math. Soc.*, **35**, 643-683 (1929).

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TWENTY EXACT FACTORIALS BETWEEN 304! AND 401!

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In the year 1944 the author published privately a little book entitled *Exact Values of the First 200 Factorials*. Subsequently he computed with great care the exact values of $n!$ from $n = 201$ to $n = 300$. The data of this third century have not appeared in print. One consultable copy has been deposited in the library of Brown University, Providence 12, Rhode Island, and another copy is in the possession of Doctor J. C. P. Miller, Technical Director of Scientific Computing Service Limited, 23 Bedford Square, London, W.C. 1, England.

Recently the author has computed a skeleton table of 42 exact factorials beginning with 303! and ending with 400!. This table was built up by first calculating the values of $n!$ for which $n + 1$ was one of the 17 primes from $n = 307$ to $n = 401$, so that Wilson's theorem could be applied as a more exacting check in addition to congruence testing with moduli such as $10^5 + 1$, $10^8 + 1$, etc. Incidentally the values of 350!, 372!, 375!, 378! and 400! as found by the author in February, 1945, were reproduced identically in the work performed three years later. In order to make a few of these arithmetical constants available to other investigators requiring exact values in the fourth century of $n!$ the following table of equally spaced but non-consecutive data is presented.

305!/10⁷⁵ =

16143	67270	06180	26842	21781	89219	57830	70329	83534	63749	47728	78
66413	96628	16717	92393	86921	72994	33010	41362	09502	42202	06171	
62425	70005	87970	32565	74046	67837	53084	59719	20375	86704	96780	
27329	65215	01067	57207	75248	99979	71751	10374	28293	65958	21786	
98340	33476	64249	90189	04484	54025	12887	81096	82388	28344	65942	
17304	84960	08989	52299	90223	10631	53132	49820	70510	36643	66235	
87970	71920	65590	58389	70553	59515	39835	59467	74139	56369	78246	
12784	47724	93307	09006	35589	61199	74856	43598	64728	70357	22731	
42776	53698	93630	46708	22644	31683	43792	23082	92544	25088	11617	
53040	88347	71608	33635	71475	56100	82949	78164	20479	73459	23072	

310!/10⁷⁶ =

57382	46323	52647	78187	95476	79518	99579	52188	49117	49141	81516	2166	32307	39615
69177	68056	63364	68240	67318	71222	58715	50633	17192	53555	64906			
02511	56380	44857	87856	98918	43414	84630	74133	24707	51131	32472			
66239	23677	47417	72624	82291	59184	42537	62262	89172	60707	31456			
57018	79241	49987	04985	83771	09316	72970	22939	23879	45276	93358			
86726	60161	75252	63987	11567	16646	56686	20269	46552	69368	82261			
63413	89016	78640	80356	86331	68719	62066	19987	30510	47909	31513			
70148	32280	60402	28659	87648	08466	70375	93175	75294	06975	27745			
32860	60968	62580	63385	63798	61981	33889	16877	69791	67062	98763			
90059	94763	57218	32760	86222	26812	05438	36586	57430	21171	01568			

315!/10⁷⁷ =

91280	84648	67246	93577	25980	02786	05076	28432	35846	76142	84900			
08722	14393	27856	51973	27268	67012	22354	17592	68574	72307	60553			
61061	94568	15248	53610	41430	89351	01368	99668	13922	65197	62140			
64139	40839	25901	58615	43169	46069	44417	78372	98555	22524	09073			
81753	22134	92158	19852	24245	53131	19258	22462	95412	09823	69427			
31544	79121	68349	69260	74271	29127	63153	61009	53702	68216	65849			
57188	45236	83646	81102	37278	43235	51317	63211	48729	06486	79100			
13746	83046	89399	57977	04211	22719	41248	51953	75150	99839	36963			
32916	48221	19039	16368	62309	74000	23742	66148	22088	25999	10187			
80899	51827	27950	77930	26927	27664	80616	94761	43746	36373	93408			

320!/10⁷⁸ =

87946	39108	37613	06765	21	16103	34721	92524	82955	71704	10776	29865		
08317	09407	22591	24180	71083	82859	29587	26413	48645	16639	12600			
40395	58390	89805	02774	34785	61543	14632	61485	73930	87562	33136			
98969	64916	31377	72782	92965	20278	06263	04839	72525	43230	83321			
24593	59203	45445	76046	93157	16688	80818	13860	83935	73770	52843			
53395	86952	08617	42156	12749	63850	90743	60230	90498	20934	91713			
47554	61873	01294	57049	38955	13272	46630	75880	43699	59040	93654			
70934	95526	56965	61054	65403	72048	42102	66089	25808	49397	81640			
19986	59344	25649	05462	74566	94123	26023	29181	22696	08558	33215			
77599	89142	54964	92653	59278	84808	48689	20655	69846	12424	25344			

325!/10⁸⁰ =

38059	74	39241	74558	20825	43735	34494	22105	61663	83967	17520			
54750	07317	38414	66417	00760	47025	41857	66473	78675	82293	78970			
93161	30667	62898	76155	84867	98163	29339	93348	42298	37744	03857			
16423	46480	42003	51689	40232	91722	46643	76150	36938	24510	20933			
57687	02926	22957	07373	81260	20152	52321	88823	20676	72634	49792			
01190	87656	32541	67677	40192	65463	74562	08691	55586	99269	82630			
22078	29558	78973	52044	43614	38588	21524	87400	48531	73763	00573			
81490	77271	50660	94791	24466	15706	93170	75740	94166	74152	78143			
37831	07929	38952	08733	37686	10203	73826	49092	43130	35525	30809			
74862	71472	66624	77638	86024	68739	41043	51267	64486	39822	27540			
	41909	29892	71284	09589	30646	07043	81637	21506	68598	96832			

3301/10⁸¹ =

											2824
08462	25838	71086	56010	65535	09844	21358	23061	55484	47917	12597	
38190	61204	08795	34106	75131	67173	45648	85871	83699	48575	84240	
51605	24086	04307	34116	50670	52618	23551	53355	92584	91538	01248	
88501	44424	17647	15270	10285	80624	04841	10526	90725	30716	90361	
23418	80567	81083	95522	57654	49360	11494	92226	04307	54462	86770	
76900	02535	50746	72950	34249	73082	81617	04937	16827	30704	92544	
57005	43334	27097	51252	77956	18877	50731	81736	31303	45796	96036	
35006	71701	03798	94122	54276	57240	69914	28638	65586	03964	57246	
35073	71484	51362	95430	04002	37985	66203	76408	67840	59147	34449	
85051	11170	69154	59551	70513	50965	81824	24889	07209	26775	93945	
62763	90199	87779	97874	88537	30433	82846	84150	35962	15208	50944	

3351/10⁸² =

											1
93897	53538	04080	10912	68491	24676	06380	60093	13907	45734	40409	
72220	62840	52553	61624	80175	28415	75881	72507	37601	10680	52800	
94872	50352	03705	25004	15910	40543	98239	92655	53881	48596	22741	
10477	33655	22467	39523	54575	91597	72316	07882	10176	61380	54718	
92395	28832	63254	05333	46598	01513	83359	87995	14906	97445	91206	
79882	41487	93034	03739	47780	88438	44582	06602	87501	19045	25452	
34348	98810	30684	78921	64591	78597	76099	81759	32112	25628	97869	
32411	05858	98441	58812	31372	54159	30812	86923	47611	05015	13791	
81839	45036	48165	36811	00870	74766	75969	31540	60988	93507	74705	
20248	94533	26775	83896	60107	30186	90638	57812	71218	27780	08281	
48363	62059	19007	07450	13954	58751	46455	28828	73065	19947	50976	

3401/10⁸³ =

											51
39272	93363	03446	71982	88537	60394	68582	43798	11307	08498	58229	
21313	63729	54649	89902	03510	11761	05977	88061	33788	93649	35774	
34500	36168	34151	88982	47240	13463	38884	86114	14227	40713	13888	
05908	33811	29369	42713	65431	21020	13237	23370	85743	88954	02320	
41192	95061	83969	59886	33829	52818	38936	92358	32500	91164	12814	
17808	59964	38696	88726	98869	90263	12600	54113	91824	90611	59323	
94760	45067	55237	53157	38712	39151	79975	20501	62388	22164	26473	
86346	85875	38818	06087	52182	40504	10520	57069	47492	36518	81064	
25431	86264	10104	74387	42041	85541	64289	64941	04086	29854	29878	
77862	04180	85044	53671	65509	33980	52608	36237	88608	80585	11128	
06177	13156	22325	53426	32769	29061	01835	27477	35327	20140	12416	

3451/10⁸⁴ =

											2421
20556	04025	86527	34839	78326	70399	61720	17832	35931	74739	04791	
36170	79695	53150	26894	73012	21382	08891	34885	85399	28184	38056	
44508	02014	82863	67524	04948	02269	82311	01258	81000	28468	73771	
04376	40079	22001	65127	85590	84980	47507	34795	54466	03093	96432	
69870	87311	39427	46842	37308	39850	29113	04969	71971	50980	68025	
49750	49007	30580	21701	65732	70011	69846	73789	24291	55078	08736	
05154	73687	95426	02554	63555	84282	65690	30209	13423	59471	86350	
86275	16511	20347	83535	42187	15104	58382	67239	16892	87475	25890	
55970	84876	55213	48872	75308	84968	55871	63850	00436	98912	94795	
27833	01034	05177	60688	34536	87157	29020	01533	68625	34353	87691	
48712	01776	69920	58786	62858	55585	72655	44230	99917	84492	56448	

$350!/10^{10} =$

	12358	74058	26548	87501	43951	99786	54645	72245	32073	94691
95158	79429	33023	00930	35357	49131	42169	34583	29501	11784	45941
55210	94327	61532	44976	77618	92237	04344	49422	13964	09009	16694
90545	66125	51113	34533	06982	54556	07852	78983	64515	85122	90209
96499	77304	22679	48748	40601	81101	77641	37584	86813	75049	75397
32592	58825	41777	11770	66194	90238	36340	92545	89994	07933	46268
93194	60801	68889	86949	68499	43334	59029	36521	45557	84862	35393
91025	67266	74571	28468	24819	00414	60641	84543	88812	35334	64975
62117	92870	75018	58648	13576	43313	07515	33590	02713	29461	16326
14208	13403	66501	16689	05258	55733	50955	36024	61704	51786	97235
13653	70405	72203	62943	85680	47828	72788	27977	51141	19090	71460
91480	76811	31728	23218	29915	17416	47048	31579	98067	48729	01632

 $355!/10^{10} =$

									6	77376
79776	67418	36099	91145	74273	79142	17542	22965	94923	96674	85209
67948	42281	92577	25475	68718	76618	99834	45933	83880	00852	20067
66859	41701	64400	46981	51667	73291	99631	37983	30499	20048	47652
69211	33921	98225	85634	71407	45050	89179	92648	79045	82804	53482
95329	34402	31039	32710	67286	29446	84060	92412	17643	10350	09354
15071	36870	09762	70611	86515	42496	65836	80466	48264	70890	83152
52700	86372	02398	72164	48607	98604	82079	83395	29533	76062	21827
31586	53268	75385	27004	06698	59604	94844	22827	62427	33819	36514
84358	18140	02625	83842	48233	15155	34787	84090	78490	00319	93652
86972	90882	53919	73620	37005	91070	19241	32274	07960	77548	20804
63229	42413	38087	58738	22495	80491	18413	82129	64553	75338	50288
82630	03835	65229	48801	11161	99399	03669	44277	57585	80366	70464

 $360!/10^{10} =$

							398	31669	22118	81067
82059	90336	56471	84342	24120	60566	41437	01183	60876	81419	05250
78778	28777	19783	67867	90614	84962	36508	15370	98035	92350	12970
45164	32578	31407	44700	98769	49575	32389	15840	08581	16442	93357
64558	98753	75992	53484	56032	60865	02781	50327	11018	08348	16760
59630	30972	86523	60528	04284	29436	10644	52529	81359	91037	79308
18774	85511	24524	74332	24987	34122	59136	13236	87319	17415	05398
32960	42291	39669	14391	50433	27960	03064	68690	30670	76824	89567
38775	70873	54859	06504	25633	44537	36488	02120	53587	26905	56957
48062	64667	48658	11939	84051	00909	71364	83506	52527	17494	84080
03573	38638	70881	76356	70004	57650	97378	45399	27240	20434	42156
82704	41950	38802	61322	96257	83142	49284	57061	97046	20700	40415
51342	09534	71940	89863	85965	92833	35973	44225	59404	29503	52896

 $365!/10^{10} =$

						25104	12867	55587	32292	92044	37488
12027	70516	55202	69876	07976	68725	95193	90110	61382	20937	41966	
60180	09000	25416	93761	72314	36098	23286	60708	07112	33699	79853	
44536	79106	53872	38359	97043	55532	74093	76780	91491	42944	08643	
18046	92507	45101	34847	02554	60140	98005	90796	55410	41195	49010	
53118	86173	37343	51455	17193	28276	08477	55882	29169	02135	39123	
47918	62747	01519	39680	85049	40722	60703	30012	46328	39880	05504	
87427	99987	66904	16973	43786	10781	85344	66796	68715	11049	65388	
81301	36836	19901	05291	80056	12584	45494	88648	61768	29168	26347	
56414	89909	84138	06780	99996	04687	48814	67348	37340	69935	98387	
91124	99595	78845	38873	61666	15330	93253	55125	68450	56046	38873	
81297	02951	38115	18614	13688	92298	65100	05440	94394	30146	99244	
11255	57552	79140	76049	27642	53740	25041	03910	56421	97900	32896	

370!/10⁹⁰ =

		16	94213	78414	97677	14980	34360	30075	05751	79884
98526	65620	28567	19083	85739	75834	14630	44271	28457	69099	10556
45734	38389	63692	07629	59730	20538	52992	49859	15172	53291	18045
50605	24925	12753	30648	39291	55304	30725	44244	10170	09622	13277
09589	01110	21672	66208	37640	86326	80159	57769	37938	31169	49085
43218	54848	49734	38746	43632	33052	95793	30285	23312	39633	71425
93072	08890	73797	30245	28315	33903	67363	61090	94054	89619	62687
25563	17835	69154	28332	14179	68564	19238	71762	37187	65861	86085
92976	50059	48941	77965	90930	14447	02879	34628	00869	38422	66265
97744	04705	76318	83892	17541	82866	40801	14234	62726	22278	91895
20636	70305	45494	54203	49190	38529	63458	96040	54050	49820	43983
29354	26972	35900	42264	39512	45608	55397	99212	39031	45458	28703
98306	38826	37414	73701	87610	64076	40553	65686	99003	71078	67648

375!/10⁹⁴ =

	12	23197	99935	77775	43039	69618	24144	35162	61034	26817	76910
64536	25695	77925	77806	60759	41039	92280	46573	18461	01687	26782	
25710	03107	94190	68074	39962	29568	36014	52548	06731	60842	01910	
45075	25971	40041	19483	63857	80515	18320	80524	58185	11336	63561	
23602	82539	94656	12094	59748	20721	31334	17742	46378	34418	90092	
84130	44918	22114	02760	68740	11239	08686	25321	07007	62476	70111	
36150	01670	11391	80232	70088	04755	53111	90619	92226	05070	32665	
60109	19117	74798	82909	32490	49834	50739	53856	35116	54387	25712	
59963	95422	98301	58661	85582	58435	62114	04943	50085	91617	82334	
29034	09063	68839	74842	74664	42123	43086	33728	07958	39810	61102	
13513	29535	61148	81688	64444	48735	72596	82637	63093	53951	75676	
92235	76659	26676	72909	77647	15873	71794	82824	68011	07939	82003	
05658	02166	43741	06889	75365	67417	77488	54283	88049	65564	08832	

380!/10⁹⁴ =

									943	93168
35166	58867	93554	22817	58299	37281	47585	04032	64861	53629	48674
40001	07042	45127	34225	96890	47843	36560	50455	91347	30512	88470
75730	02822	59578	82791	11896	52265	92961	83926	70347	76856	57760
56456	17888	00392	69308	34123	98664	31499	07809	85234	90078	50619
01304	07985	25252	56361	35193	78619	88290	90641	81536	23632	16117
88837	69938	62150	25314	15986	81319	25331	98006	26643	01149	51917
98466	10485	92587	82855	56858	32339	61379	27770	97590	40186	50227
34476	03796	54358	27575	69195	31413	89397	53825	48193	35594	77692
29240	61513	44190	06943	60283	76253	31360	91446	21004	49102	41971
05636	06579	58334	60770	92618	33655	68594	53667	19124	82069	97050
21756	00464	15900	27865	01236	44293	05991	91665	93324	44813	26462
29613	54191	86241	84153	20160	21543	17437	56899	04305	31490	17526
76716	52093	95529	48051	22901	49804	42638	97333	46260	00976	54784

385!/10⁹⁸ =

						77789	25820	00226	82857
26252	46114	15309	14984	70558	30033	03410	98826	89650	25219
79085	09462	50041	60614	77714	28400	32749	70096	00180	16880
06007	60973	38706	36254	65151	97397	79798	79777	23879	66375
85963	69895	63488	39607	93900	53459	32156	76317	53747	63295
54510	91172	33552	74009	75225	03021	67318	74738	07397	90814
88674	48117	85875	77495	62590	48468	26489	29702	78702	22627
02072	86421	81787	64018	22851	01009	79403	76433	86965	05089
88419	21622	59601	87559	78614	76842	62118	67938	80884	77835
22973	15903	45851	74157	43852	45890	02638	31761	20681	01579
06181	42271	52746	58208	74530	48037	97654	91556	99326	58041
47419	61323	60265	36061	94370	32037	02535	56826	46278	40497
05384	86783	77919	14952	61331	72785	58824	65027	08157	48037
31197	62669	48667	91618	77759	74266	35292	58702	75040	92581

• $390!/10^{96} =$

49476	31481	32747	70285	35066	40121	77738	22066	23906	18964	87020
15813	55764	00709	57506	24122	30994	20282	47435	90161	45630	25958
69091	32612	29689	90881	16302	04905	80621	16147	86597	15395	88944
37690	74225	18614	81190	22832	22676	97055	74128	29408	11292	44511
90144	16657	12949	47309	19174	48139	32033	97102	62195	68770	51353
41665	36533	05575	23282	74776	27056	92163	10894	64050	24495	34064
52641	73808	10191	99624	89292	31724	79753	76876	73260	90076	09292
52617	27008	63082	87559	48519	52005	44928	91329	28666	83679	53139
37443	76458	15544	65013	44573	00695	13994	81916	58099	62058	28255
45136	32941	27280	44585	03088	65616	07954	73749	38828	02304	54827
59566	42726	25262	00294	39437	36441	24020	21295	20450	86447	84395
62773	47809	03782	04531	07276	29068	39469	39866	22008	75524	55757
36315	55379	02670	45812	69487	01877	04539	11393	43943	14467	57376

• $395!/10^{97} =$

87024	22053	6412	28070	65834	24106	22415	93125	16580	47304	96357
02485	38560	35488	52691	82750	76913	18517	38630	06440	24491	10408
65322	19290	59920	40283	83584	01757	94698	03393	34596	25781	14094
30070	64560	34908	78194	94315	43117	81988	26927	29553	14076	41555
09082	52633	11267	50827	75952	38905	10563	63700	31748	89013	91022
38531	73584	82407	31077	73540	81436	19537	08102	21335	57278	28043
65810	54163	28037	51254	21717	90143	18721	93988	19061	80759	26665
52578	97035	60708	64409	01654	02267	38728	56106	88950	88866	56182
15002	12091	79017	73406	43833	73487	87366	01446	24545	25826	37274
58417	47046	70552	84850	52979	65994	71285	27727	83551	08789	02120
08596	77481	59110	03145	42365	21007	01698	79736	04888	97935	18640
21700	90452	10565	26818	12901	25372	41430	45005	60198	08312	84886
81894	23869	06626	37443	78647	94187	84777	19873	44441	93099	28448

• $400!/10^{99} =$

64034	52284	60238	95262	34797	03195	03005	85070	25830	26002	95945
86844	45942	80239	71691	86831	43627	84786	47463	26467	62943	50575
03585	68108	48298	16288	35174	35228	96198	86468	02997	93734	16541
50838	16242	64619	42352	30704	62443	25015	11444	86708	90662	77391
49181	17331	95599	64407	09549	67134	52904	77020	32243	49112	10797
59328	07951	01545	37266	72516	27877	89000	93497	63765	71032	63503
31533	96534	98683	86831	33935	20243	73788	15778	67915	06311	85870
26182	70169	81974	00629	83025	30859	12983	46162	27230	45583	39520
75961	15053	02236	08681	04332	97255	19485	26744	32232	43866	99484
22404	23259	98055	51610	63594	23769	61399	23191	71340	63858	99653
79701	47827	20660	63202	17379	47201	03213	56624	61380	90779	42304
59736	06995	67595	83609	61587	15129	91382	22865	78579	54936	16176
54480	45322	20078	25818	40084	84364	15591	22945	42753	84803	55837
45180	22675	90006	13996	60145	59520	61272	11192	91810	50324	91008

THE DETERMINATION OF HEREDITARY ANTIGENIC DIFFERENCES IN GENICALLY IDENTICAL PARAMECIUM CELLS*

BY T. M. SONNEBORN†

DEPARTMENT OF ZOOLOGY, INDIANA UNIVERSITY

Read before the Academy, † April 26, 1948

Analysis of the determination and inheritance of antigenic traits in the unicellular animal, *Paramecium aurelia*, shows that cells which are identical in genic constitution may yield, under identical conditions of culture, progeny that are persistently diverse with respect to these traits, in spite of the fact that determination of the traits is ultimately traceable to the action of genes. As comparable persistent diversities among cells presumably identical in their genes arise normally in the development of higher organisms, the mechanisms discovered in *Paramecium* may be of general significance.

The present paper is the third of a series setting forth results obtained in a study of variety 4 of *P. aurelia*. The first two papers,^{1, 2} in which the earlier literature is reviewed, may be briefly summarized as follows. In stock 51, four antigenically diverse hereditary types (A, B, C and D) have arisen; each of these is immobilized only by its corresponding dilute antiserum, not by dilute antisera against the other three types. Exposure of animals of any one of these types to its immobilizing antiserum for a short time is followed by transformation of antigenic type in high proportions of the exposed animals. These changes are from one to another of the four types, A, B, C and D. The transformed animals yield cultures that remain permanently true to their new type through both vegetative reproduction and fertilization. When interbred, the four antigenic types (both those that arise spontaneously and those that result from experimental transformations) manifest no segregation in the F_2 generations, indicating the absence of gene differences among them. Antigenic type strictly follows the cytoplasm, indicating its cytoplasmic control. Other points and the interpretations will be deferred until after the new data have been presented.

New Materials and Extension of Previous Results to Them.—Two new antigenic types, E and G, have arisen in the same stock 51 and immobilizing antisera have been prepared against them. A few cultures do not react to any available antisera, so one or more antigenic types remain to be identified. In another stock (No. 29) of variety 4, six diverse antigenic types have been isolated and identified and one or more remain to be investigated. Of the six known types, four are immobilized by the same antisera that immobilize types A, B, C and D, respectively, in stock 51. They may therefore be designated by the same symbols, though there is evidence that at

least some pairs of corresponding antigenic types in the two stocks are not absolutely identical. Thus far, no culture in stock 29 corresponds to type E or G of stock 51. On the other hand, two types, F and H, against which immobilizing antisera have been prepared, have arisen in stock 29 and thus far no culture in stock 51 corresponds to them.

All the new antigenic types (E, F, G and H), as well as the stock 29 homologs of types A, B, C and D, have been exposed to immobilizing antisera, in the way previously reported^{1, 2} for types A, B, C and D of stock 51, in order to ascertain whether hereditary transformations of antigenic type could be induced. Using this method, large proportions of the animals (sometimes up to 100%) of every one of the types in both of the stocks can be transformed to other types. All transformations induced by exposure to antiserum fall within the spectrum of types characteristic of the stock employed (A, B, C, D, E and G in stock 51; A, B, C, D, F and H in stock 29). Even within these spectra, however, there are curious limitations. For example, in stock 29, Type D seldom transforms directly to Type F after exposure to anti-D serum; but it readily transforms to Type B which in turn can readily be transformed to Type F by exposure to anti-B serum. Any type in the stock 29 spectrum can be transformed to Type F by not more than two steps. Moreover, the transformations are reversible. Thus, if Type A of stock 51 has been transformed to Type B by exposure to anti-A serum, the resulting Type B cultures can be transformed back to type A by exposure to anti-B serum. As with transformations to Type F, reversals of type may sometimes require a two-step transformation. Transformation thus involves no complete loss of any antigenic possibility.

Interconvertibility of the types within a stock shows that each type possesses, in addition to the antigen that characterizes it, the capacity to produce the antigens characteristic of all the other antigenic types within the spectrum of the stock to which it belongs. Each hereditary antigenic type has therefore been defined^{1, 2} as having a primary antigen, the kind involved in its immobilization by antiserum, and a number of secondary or potential antigens, the remaining kinds in the spectrum of the stock to which it belongs. One of the secondaries becomes the primary when transformation occurs spontaneously or is induced. In the earlier papers^{1, 2} the various secondaries were held to be present in relatively small amount because relatively small amounts of specific antibodies against them were frequently detected in the antisera. In subsequently obtained antisera, however, antibodies against secondaries have been detected less frequently. While presence of antibody in a serum is evidence for presence of corresponding antigen in the paramecia used for injection, absence of antibody in the serum is not sufficient evidence for absence of the corresponding antigen. Secondary antigens appear therefore to be at least sometimes actually, and always potentially, present.

Control of the Direction of Transformation.—Our previous papers^{1, 2} reported no control as to which new type or types would arise following exposure to antiserum. Theoretical considerations, set forth below, led to testing the possibility of *directing* transformation by combining exposure to immobilizing antiserum with exposure to certain other conditions, of which only diverse temperatures and amounts of food have thus far been explored. The results were dramatic. Decrease of temperature and decrease in amount of food have similar effects and together they have effects greater than either has alone. The full results, carried out on all 12 type-stock combinations with temperature and food varied separately, will be presented elsewhere. They are well illustrated by the following example. When Type D of stock 29 is exposed to anti-D serum, 94% of the animals transform to Type B if exposure and subsequent growth for a day or two are carried out at 32° in the presence of excess food; but 96% transform to Type H if the same experiment is carried out at 20° with enough food to permit only one fission per day. Similar control of the direction of transformation has been achieved with the other antigenic types.

In view of these results, further theoretical considerations led to the prediction that similar directed transformations should be possible, but more slowly, by employing diverse temperatures and food supplies, without subjection to immobilizing antiserum. Tests fulfilled the prediction. Extensive experiments, which will be presented in full elsewhere, are well illustrated by the following examples. In stock 51, without exposure to antiserum, at 32° Type A remains constant, but types B, C, D and E all transform to Type A; while at 12° Type B remains constant and other types transform to Type B. The transformations at 12° are slower and less regular than transformations at 32°. The effect of food supply is shown conspicuously by animals of Type C in stock 51, which, at the standard temperature of 27°, remain constantly Type C if provided daily with enough food for only one fission, but transform to Type A if provided with an excess of food. Our earlier difficulty² in maintaining stable cultures of this type resulted from providing them with too much food.

The facility with which antigenic types can be transformed by exposure to antiserum, to diverse temperatures and to diverse food supplies (perhaps also to other as yet untested conditions) is in marked contrast to the stability of all the types, regardless whether of spontaneous or induced origin, under standard conditions of culture at 27° with enough food added daily to permit one fission. Under these conditions, each type, regardless of origin, is hereditary, maintaining itself constantly through both vegetative and sexual reproduction.

Relation of Cytoplasmic to Genic Factors in Determination of Antigenic Type.—As set forth above, diverse antigenic types in the same stock are alike in genes and diverse in cytoplasm. The question arises as to whether

the antigenic differences between stocks are determined in the same way as antigenic differences within a stock. The one stock difference thus far analyzed is the capacity to manifest antigenic Type F, which exists in stock 29, not in stock 51.

The two stocks were crossed and the F_1 generation was allowed to produce an F_2 generation by autogamy (self-fertilization in unpaired animals). The antigenic types appearing in F_1 and F_2 are not in themselves of primary interest and those details will be presented elsewhere. Here we are concerned primarily with whether each F_2 clone could or could not be transformed to Type F (if it was not initially Type F), when subjected to the conditions known to favor transformation to that type. Half of the F_2 clones were Type F or were transformable to Type F and the remaining F_2 clones could not be induced to yield Type F. This 1:1 segregation in the F_2 occurred regardless of whether they were derived from F_1 clones with stock 29 cytoplasm or with stock 51 cytoplasm and regardless of whether there had or had not been exchange of cytoplasm in the initial cross between the two stocks. Apparently, therefore, the results are independent of F_1 cytoplasm. On the other hand, a 1:1 ratio among clones obtained by autogamy is the result required³ when dealing with segregation of one pair of allelic genes.

The two classes of F_2 clones were then backcrossed to the parent stock of opposite class and autogamy was obtained in the backcross clones. Both sets of experiments yielded again the same 1:1 segregation. For the three experiments together, the totals were 86 clones capable of yielding Type F and 95 clones incapable of yielding Type F. The capacity to yield clones of Type F thus depends on a single chromosome, and presumably a single gene, present in stock 29 and lacking in stock 51. This gene determines not the realization of Type F, but only the capacity to realize it under definable conditions. If the other antigens show similar mechanisms, it may be concluded that the genes control the antigenic possibilities, while the cytoplasm controls which of the possibilities is realized and perpetuated.

Summary and Interpretations.—(1) The antigenic types are hereditary under standard conditions. (2) Diverse antigenic types within a stock are alike in genes, but differ in cytoplasm. (3) Capacity to develop antigen F depends upon a gene; hence, cytoplasmic control of antigenic diversity within a stock is ultimately gene-dependent. (4) Hereditary transformations within the spectrum of antigenic possibilities of a stock are readily brought about by environmental conditions (specific antiserum, temperature, food supply). (5) Approximately 100% hereditary transformation in either of two alternative directions may be produced by specifiable conditions; hence, the gene-dependent cytoplasmic control of antigenic diversity within a stock is also environment-dependent. Unlike the well-known examples in higher organisms of alternative phenotypes being de-

pendent upon the conditions during development, the effects of external conditions on the phenotype in *Paramecium* persist throughout all subsequent generations, asexual and sexual, under standard conditions.

The foregoing relations may be interpreted on the hypothesis that each antigen is, or is determined by, a plasmagene producible under the action of a gene or genes; and that the diverse plasmagenes compete with each other, the competition being influenced by environmental conditions and by the relative cellular concentrations of the various plasmagenes. On this hypothesis, the constancy of all antigenic types is due to the multiplication of all the plasmagenes at the same rate of one duplication per day under standard conditions of culture (i.e., the rate of cell multiplication under these conditions). Antibody merely facilitates transformation by suppressing or retarding multiplication of the antigen with which it combines (see Beale)⁴. Then one of the other plasmagenes, whichever is best adapted to the prevailing conditions, replaces the suppressed one. Transformations following cytoplasmic exchange at conjugation are set in motion as a result of lowering the concentration of the original plasmagene and raising the concentration of the acquired plasmagene, the change in proportions providing a new competitive situation.

One of the main alternatives to the plasmagene hypothesis is an hypothesis of variable gene activity⁵ in which the activity of each antigen gene varies directly, up to a certain limit, with the cellular concentration of the antigen it controls. To fit this hypothesis to the fact, however, a number of improbable assumptions are required. Moreover, when control of the direction of transformation was achieved on the basis of predictions suggested by the plasmagene hypothesis, a further improbable assumption had to be made to reconcile the new facts with the variable gene activity hypothesis. Although further critical tests distinguishing between these hypotheses remain to be made, the relative simplicity and reasonableness of the plasmagene hypothesis, together with its proved predictive value, justify its tentative adoption.

Relations to Other Work.—Evidence for gene-initiated plasmagenes in variety 4 removes the main difference previously held⁶ to distinguish two groups of varieties of *P. aurelia*. Of the three kinds of traits—killer, antigens and mating types—that show cytoplasmic control in variety 4, two have now been analyzed: in one (killer trait) the cytoplasmic factor is *not* gene-initiated; in the other (antigens) the plasmagenes *are* gene-initiated. Counterparts of both mechanisms are in the literature: no evidence of gene-initiation has been reported for the CO₂ “genoid”⁷ in *Drosophila* or for the plasmon⁸ of plants; but gene-initiation of plasmagenes is indicated⁹ for adaptive enzymes of yeast (see also reference 10). The large size and staining properties of the killer cytoplasmic factor kappa are unique¹¹ among the cytoplasmic factors in *Paramecium*; the failure of genes to

initiate it may likewise be unique. At least, gene-initiated plasmagenes can be more readily assimilated into current genetic knowledge and theory which demand ultimate gene control of hereditary capacities.

The cells of a multicellular organism are like the cells of a clone of unicellular organism in their presumed identity of genes. Nevertheless, in both the Metazoan and the Protozoan persistently diverse types of cells arise. In *Paramecium*, our analysis indicates that persistent diversity of traits may arise as a result of environmentally produced quantitative differences among a series of alternative plasmagenes. The same mechanism may therefore be suggested as the basis of cellular differentiation in higher organisms. If this turns out to be correct, control of cellular transformation in tissue cultures and in normal and abnormal development is indicated, and genetics has moved a step closer to its necessary ultimate fusion with experimental embryology.

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‡ Under the title: "Genes, Cytoplasm, and Environment in the Control of Cellular Heredity."

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THE PROCESS OF TRANSFORMATION OF ANTIGENIC TYPE IN *PARAMECIUM AURELIA*, VARIETY 4*

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Introduction.—The diversities in antigenic type in *Paramecium aurelia*, variety 4, have been shown to be purely cytoplasmic in heredity (Sonneborn^{1,2}), provided that attention is confined to animals of a single stock,

derived from a single wild ancestor. Since antigenic types breed true under certain conditions, continuing unchanged through both asexual and sexual reproduction, there must be some mechanism in the cytoplasm for the reproduction of the antigens or of their precursors. In this investigation an attempt is made to gather some data relevant to the problem by making a quantitative study of the antigens in paramecia under varying conditions, particularly during transformation of one antigenic type into another.

Measures of Antigens and Antibodies.—It has been shown by Masugi³ that paramecia, when in sufficient quantity, are able to remove the paralyzing antibodies from an antiserum, rendering it ineffective against any fresh animals. The ability to absorb antibody from a standard solution may therefore be used as a measure of the antigen in an animal.

As a measure of antibody, the time taken for a paramecium to be immobilized when placed in a large excess of solution containing the antibody, can be used. Data in table 1 show that the reciprocal of the immobilization time is roughly proportional to concentration of antiserum for solutions immobilizing in 20 minutes or less, but that with weaker solutions, the immobilization time increases more steeply. Within a stock, the immobilization time, under standard conditions, is uniform; but animals from different stocks, though paralyzed by the same antiserum, may have significantly different immobilization times with the same concentration of antibody.

TABLE I
RELATION BETWEEN IMMOBILIZATION TIME AND CONCENTRATION OF ANTISERUM D-47
AT 25°C.

DILUTION	IMMOBILIZATION TIME (MINUTES)		
	4	5	4
1/200	10	10	10
1/400	21	22	22
1/800	53	70	50
1/1600	>120	>120	>120
1/3200			

Each column represents a different sample; and each figure represents mean of 10 observations.

Some preliminary experiments were made on the rate of absorption of antibodies by paramecia. A known number of animals was placed in a given volume of suitably diluted antiserum, samples were removed from time to time, and centrifuged. The strength of antibody in the centrifuged sample (i.e., in the supernatant) was determined by putting a few fresh paramecia into it, and comparing their immobilization times with those of animals put into unabsorbed antiserum. The amount of antibody absorbed by the animals, expressed as a fraction of the amount originally present in the solution, could be then estimated. Table 2 shows some typical results.

It is seen that the rate of absorption is rapid at first, but soon slows down; and that the amount finally absorbed is roughly proportional to the number of animals.

Measure of Antigens during Transformation.—The antigenic type has been found to remain constant if the paramecia are provided with sufficient food for only one fission per day at 27°C. (Sonneborn⁴). Under these conditions the rate of production of antigens parallels the growth rate of the animals. During transformation of one type into another, however, there is a loss of one antigen and a simultaneous gain of a new one, and therefore the transformation period is a profitable time for the study of antigen production. As described by Sonneborn^{1,2} transformation of Type D (stock 51) to Type B, is readily accomplished by treating the D animals with their paralyzing antiserum, subsequently placing the animals in culture fluid, allowing them to recover and pass through a few fissions.

TABLE 2

ABSORPTION OF ANTIBODIES FROM SERUM D-47 (DILUTION 1/500) BY TYPE D PARAMECIA

ABSORPTION PERIOD (MINUTES)	1350 ANIMALS PER CC.		4050 ANIMALS PER CC.		12,150 ANIMALS PER CC.	
	(1)	(2)	(1)	(2)	(1)	(2)
0	11	0	11	0	11	0
15	19	0.42
30	16	0.31	26	0.58
60*	12.5	0.12	16.5	0.34	30	0.63*
120	17	0.35	21	0.48	50	0.78*
180	16.5	0.34	20	0.45
300	17	0.35

Explanation of columns: (1) Immobilization time (minutes) of samples of solution after absorption. (2) Calculated proportion of antibody removed from solution during absorption assuming inverse linear relation between concentration of antibody and immobilization time.

* Owing to long immobilization time, these figures are approximate only.

It was first of interest to determine whether the serum treatment directly produced the transformation—i.e., whether paramecia on being removed from the serum (after treatment for two hours or less) had lost their D-type antigens and replaced them with B-type ones. This was found not to be so. Animals which had been immobilized by anti-D serum (though not with such a severe dose as to prevent subsequent recovery), could still absorb D-type antibodies from a solution, though to a lesser extent than normal D animals, as shown by the following experiment. The immobilization time of antiserum D-47 at dilution 1/800 was found to be 13 minutes. After absorption by 40,000 D animals for 20 minutes in 2 cc. of this solution (immobilizing all the animals), all detectable antibodies were found to be removed from the solution. The 40,000 immobilized animals, after being washed, were then placed in a fresh 2 cc. serum and after 30 minutes

had increased the immobilization time of the solution from 13 to 16 minutes, thus demonstrating that immobilized animals were still capable of absorbing antibody. After further washing and placing in fresh 2 cc. serum, the 40,000 animals were finally incapable of absorbing any more antibody in two hours. Thus the immediate effect of the serum treatment was to reduce the capacity of the animals to absorb anti-D antibodies.

It is not however necessary to immobilize the paramecia in order to bring about the transformation $D \rightarrow B$, for even a mild serum treatment, sufficient only to effect a slight retardation of the animals, yields a high degree of transformation. With animals thus treated, it is easy to test their reaction to both anti-D and anti-B sera immediately after treatment, before recovery has taken place; such animals have always been found to be immobilized by anti-D but completely unaffected by anti-B serum. Thus immediately after treatment there are no signs of the impending transformation.

Transformation of antigenic type takes place only when a period of growth follows the serum treatment. A number of stages may be recognized in the transformation process, as follows:

1. Paramecia immobilized by anti-D; unaffected by anti-B (normal D animals).
2. Paramecia immobilized by anti-D; retarded by anti-B.
3. Paramecia retarded by anti-D; retarded by anti-B.
4. Paramecia retarded by anti-D; immobilized by anti-B.
5. Paramecia unaffected by anti-D; immobilized by anti-B (normal B animals).

The length of the various stages depends upon the strength of the initial serum treatment, i.e., the stronger the treatment, the sooner transformation begins and ends. Thus in one experiment paramecia of Type D were subjected for half an hour to anti-D serum diluted 1/800, which thoroughly immobilized the animals. After treatment the animals were washed and immediately put into culture fluid. With this treatment, stage 1 lasted until about five hours after the end of the treatment, and the whole process of transformation was substantially complete in 15 hours. By this time all the animals had passed through one fission, and about one half had passed two fissions. The number of clones transformed after 15 hours was 59 out of a total of 94; further growth for 12 hours (i.e., 27 hours in all) resulted in changes in only two more cultures, which produced mixtures of transformed and untransformed animals.

With a much weaker treatment however (half an hour of anti-D serum diluted 1/6400), stage 1 lasted a minimum of eight hours, and by 15 hours only 6 out of 76 cultures had progressed as far as stage 4. But after 27 hours, a further 29 cultures had produced transformed animals. Thus, with

the weak treatment, the time at which transformation of individual animals began and ended was much more variable than with the strong treatment, though almost always later.

The necessity for active metabolism during transformation was demonstrated by placing serum-treated animals in boiled tap water for five days. Animals which had been given a weak serum treatment (though sufficient to induce transformation in animals subsequently fed and grown), did not transform at all when kept in boiled water. A stronger serum treatment, followed by starvation in water, resulted, after 20 hours, in the development of animals which, while still liable to immobilization by anti-D, were at the same time more or less retarded by anti-B serum. Starved animals, however, even after five days, never lost their reactivity to anti-D. Presumably the starved animals contained enough reserve materials to allow a limited metabolism, sufficient for partial transformation.

TABLE 3
EFFECT OF STARVATION ON TRANSFORMATION

TREATMENT	TRANSFORMATIONS AFTER 0 TO 8 FISSIONS					
	ANIMALS GROWN IMMEDIATELY AFTER TREATMENT		ANIMALS STARVED 24 HOURS, THEN GROWN		ANIMALS STARVED 5 DAYS, THEN GROWN	
	TRANS-FORMED CLONES	UNTRANS-FORMED CLONES	TRANS-FORMED CLONES	UNTRANS-FORMED CLONES	TRANS-FORMED CLONES	UNTRANS-FORMED CLONES
1	29	0	19	10	9	21
2	30	0	14	16	8	22
3	28	2	10	18	2	28
4	14	13	2	28	0	30

Treatments: 15,000—51 D animals in 1 cc. culture solution plus the following amount of D-47 serum diluted 1/400 for two hours: 1—1 cc.; 2—0.5 cc.; 3—0.25 cc.; 4—0.125 cc.

If serum-treated animals were starved, and subsequently fed, transformation was completed, though the proportion of transformed animals finally obtained became progressively less as the period of starvation became longer. Data in table 3 illustrate this. The most probable explanation of this gradual loss in ability to transform following starvation is a slow dissociation of the antigen-antibody complex while the animals are in water, though no direct evidence of this has been obtained.

Conclusions and discussion.—As a result of these experiments, the following conclusions can be made:

1. Treatment of D-type paramecia from stock 51 with dilute anti-D serum for two hours or less, produces animals with a reduced capacity to absorb D-type antibodies. Immediately after treatment, however, the animals can still be paralyzed by anti-D serum only, and are unaffected by anti-B serum.

2. When a period of active growth at 26° follows serum treatment, some

of the animals gradually gain an increasing amount of B-type antigen, and at the same time their reaction to anti-D serum becomes progressively weaker and finally disappears.

3. The times at which transformations from Type D to Type B begin and end are related to the strength of the original serum treatment, i.e., to the amount of antibody absorbed by the treated animals. With a strong, immobilizing treatment, transformation is complete by 15 hours after treatment (or slightly less than two fissions); but with a very weak treatment, completion of the process may vary from 15 to 24 hours or more.

4. Transformation is completed only in the presence of food.

Sonneborn^{2,4} has put forward a theory according to which antigen type in *P. aurelia*, variety 4, depends upon competition between plasmagenes. A given stock, or more exactly a given genotype, is capable of producing, under diverse conditions, any one of a number of antigen types (at least six in stock 51). The type actually selected, however, depends upon the results of the plasmagene competition.

The present results strongly support this theory. Further, the indications are that treatment by anti-D serum partially or completely suppresses the further production of D antigens, from which it would appear that the plasmagenes are the antigens themselves.

Finally, attention should be drawn to the striking similarity between the mechanism of antigen transformation here described, and the enzymatic adaptations in yeasts,^{5,6} and bacteria.⁷

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ON THE STABILITY OF NUTRITIONAL MUTANTS OF BACTERIA*

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The lactic acid bacteria are used more than any other for the quantitative determination of vitamins and amino acids. Most investigators have found that stocks of these organisms remain constant in their nutritional requirements and can be used for assay purposes year after year. Yet occasionally nutritional requirements have changed and confused assay results. For example, under the most carefully controlled experimental conditions, excellent determinations of *p*-aminobenzoic acid are not consistently obtained with *Lactobacillus arabinosus*.¹ Apparently, the requirement for *p*-aminobenzoic acid varies since it was found that cultures of this organism, which had maintained a stable requirement for more than three years, could be trained to give *p*-aminobenzoic acid-independent lines by subculturing in the presence of suboptimal concentrations of *p*-aminobenzoic acid.² Similarly, by subculturing in the presence of suboptimal concentrations of tryptophane, strains of *L. arabinosus* have been developed which no longer require tryptophane in the medium for growth.³

It was a recognition of these biological properties of bacteria which led Lloyd⁴ to qualify his discussion of the use of microorganisms for the determination of amino acids. He wrote: "Microorganisms do not always behave, generation after generation, like a standard reagent, nor need their behavior be independent of the general composition of the medium." Lloyd is correct; since bacteria are organisms they exhibit the property of variation. However, we need to know what the factors are that give rise to and establish variations in the nutritional requirements of bacteria.

Mutation.—Until the method of variance analysis was applied to the study of mutation in bacteria⁵ it was very difficult to discover the cause of an inherited change. For instance, the loss of a nutritional requirement through subculturing in suboptimal medium may be thought to be due to

the induction of the change in the bacteria by the chemical stimulus of the new nutrients in the absence of the normal nutrients.⁶ Or it may be due to selection by the suboptimal medium of "spontaneously" occurring mutants which are independent of a supply of the substance in which the medium is deficient, and which occur regardless of the presence or absence of the substance. The former hypothesis has been favored by many students of the adaptation of nutritional requirements.⁷ However, their reasons for this acceptance are usually inadequate and involve a failure to recognize that, although the mutation rate per generation of bacteria is of the same order of magnitude as that of other organisms, mutations in a growing culture are frequent because of the rapid rate of multiplication. Ivanovics and Eöllös⁸ have asserted that pantothenic acid-deficient strains of a *Ruhrbazillus* are variants not engendered by genetic defects because they can be made independent. But on the contrary, it is just this development of independence which can be examined statistically to discriminate between the two hypotheses on the origin of changes in nutritional habit.

Bacterial reproduction is clonal. Therefore a mutation occurring early in the growth of a culture will be represented at the end of growth by more organisms of the mutant type than will represent a later occurring mutation. Mutations are rare events, often occurring with a frequency of $ca. 10^{-8}$. Mutations occurring late in the growth of a culture, when there are more bacteria, will be more numerous than those occurring early. Hence, on a random mutation hypothesis we would expect to find a tremendous variation in the number of mutant bacteria at final growth among a series of different cultures. More cultures should contain small numbers of mutants (late mutations) than large numbers (early mutations). On the induced mutation hypothesis we would expect to find little variation from culture to culture since each organism present in the final growth has a given chance of responding to the testing conditions by mutation. The variability on this hypothesis should be due to sampling error. Comparisons of the variability in the number of mutant bacteria among several independent cultures with the variability among several samples from the same culture have been made and the former has always greatly exceeded the latter. In this way it has been shown that the development of bacterial resistance to bacteriophage, to penicillin, to sulfa drugs and to ultra-violet and x-radiation is spontaneous in the sense that it is not induced by the injurious agent.⁹ Likewise, the mutation of *Clostridium septicum* to a condition where it is independent of added uracil for growth, can apparently occur throughout growth in the presence of uracil.¹⁰ However, since the latter demonstration was complicated by several technical difficulties, an examination was made of the change to histidine independence by a mutant of *Escherichia coli*.

This mutant (No. 148-334) was secured after x-radiation at the Stamford Laboratories of the American Cyanamide Co.¹¹ I am indebted to Drs. J. O.

Lampen and R. R. Roepke who placed it at my disposal. It requires for growth salts, glucose and the amino acid, histidine. When washed cultures are plated without dilution into a chemically defined medium and agar devoid of histidine a variable and small number of colonies is formed. These upon isolation give rise to histidine-independent ($h+$) strains whose growth rate and final yield of bacteria in the absence of histidine are the same as that of the original histidine-dependent mutant ($h-$) in its presence.¹² The $h+$ strains are stable and histidine independence is inherited in the presence or absence of histidine. In this strain of *E. coli*, where gene recombination experiments cannot be performed,¹³ it is impossible to tell whether the histidine-independence that develops is due to a back mutation of the factor that makes for the histidineless character of the $h-$ strain, to a suppressor mutation or to both. At any rate, the $h+$ mutants are physiologically indistinguishable from the original wild type parent strain.

* Four separate experiments were performed to examine the variation in numbers of $h+$ bacteria from culture to culture. Strain 148-334 was inoculated into a tryptose-yeast extract medium and incubated at 37°C. for 12 hours. A 10^{-8} dilution of the resulting culture was prepared in chemically defined medium supplemented with 100 γ *L*-histidine monohydrochloride \cdot H_2O per ml. This dilution resulted in a concentration of about 35 bacteria per ml. Ten 1-ml. aliquots and one 7-ml. aliquot were placed in 12-ml. Pyrex centrifuge tubes. After growth at 37° for 24 hours the cultures were centrifuged, the supernatant decanted and the bacteria were resuspended in the original volume of chemically defined minimal medium. This procedure was carried out again and a 0.5-ml. aliquot of the final suspension was taken from each of the 10 1-ml. cultures and plated without dilution into minimal medium solidified with 2 per cent washed agar. Ten 0.5-ml. samples from the 7-ml. culture were similarly plated. To determine the total number of bacteria in each culture 1 ml. of a 10^{-7} dilution of the suspension remaining in the centrifuge tubes was plated into minimal agar supplemented with 100 γ of histidine per ml. The plates were incubated at 37° for three days and counted. The results are shown in table 1.

The variance in the number of mutant $h+$ bacteria among different samples from the same culture is always about equal to the mean number present and hence is probably due to random sampling error. In every case it is probable that the mutants were obtained from a homogeneous population. On the other hand, the variance in the number of $h+$ organisms among single samples from different cultures is many times the mean number and there is an insignificant probability that the mutants were obtained from a homogeneous population of cultures.¹⁴ Apparently mutations occur at different times during the growth of the culture in the presence of histidine. They are not induced by the absence of histidine on

TABLE I
THE NUMBER OF HISTIDINE-INDEPENDENT BACTERIA IN SAMPLES FROM DIFFERENT CULTURES, AND IN SAMPLES FROM SINGLE CULTURES

MEAN NO. OF BACTERIA PER SAMPLE ± STANDARD ERROR ($\times 10^{-7}$)	SAMPLES FROM DIFFERENT CULTURES				SAMPLE NO.	SAMPLES FROM SINGLE CULTURES			
	1	2	3	4		1	2	3	4
CULTURE NO.									
1	38 ± 2	23 ± 1	25 ± 5	43 ± 2	1	27 ± 1	32 ± 1	30 ± 3	24 ± 3
2	61	33	35	22	2	37	60	48	26
3	52	30	32	36	3	43	39	38	30
4	65	541	30	38	4	58	59	48	25
5	67	29	94	35	5	49	43	48	29
6	57	13	43	69	6	47	51	50	32
7	80	64	33	21	7	48	60	51	21
8	104	30	43	30	8	40	37	48	32
9	68	...	43	460	9	51	44	48	23
10	53	...	62	30	10	40	..	51	21
Average	103	25	...	56	..	54	27
Variance	67	106	52	77	...	47	49	48	27
χ^2	263	31,778	694	18,334	...	51	93	18	17
P	31.4	2,099	120	2,143	...	9.6	13.2	3.3	5.8
	<0.001	<0.001	<0.001	<0.001	...	0.38	0.15	0.95	0.75

the testing plates. In this respect they are spontaneous and due to unknown and possibly randomly distributed events.¹⁵

Selection.—The mere occurrence of a mutation affecting nutritional habit does not guarantee its establishment in a population. Although the mutant may have a selective advantage under certain conditions it may, under other conditions, lose out in competition with its parental type.¹⁶ Although the *h*+ and *h*− bacteria have identical growth rates when grown separately, experiments were performed to examine the possibility of competition when the two types are together in the same culture. Strains *h*− and *h*+ were separately grown in tryptose-yeast-extract medium at 37°C. for 17 hours. Then 2 ml. of the former and 1 ml. of the latter were mixed together. A tube containing 10 ml. of the same medium was inoculated with one loop of the mixture and incubated at 37°C. for 24 hours. Immediately after it was used for inoculation, the mixture of the two types of bacteria was diluted to 10^{−8} in minimal medium and 5 1-ml. samples were plated into minimal medium solidified with 2 per cent washed agar. When the plates had hardened a thin layer of the same agar medium was poured over the surface. The 24-hour growth of the mixture in tryptose-yeast extract medium was similarly diluted and plated. Three days after the plating *h*+ colonies appeared on both sets of plates which had been stored at 37°C. These colonies were counted and their position marked on the Petri dish with glass-marking ink. Then a layer of agar supplemented with 500 γ *L*-histidine monohydrochloride.H₂O per ml. was spread over the surface and the plates were incubated for another 3 days at 37°C. During this time the histidine from the surface layer had diffused throughout the rest of the plate and permitted the growth of the *h*− bacteria. These appeared as new colonies whose number did not increase after the 3-day period. Comparison of the number of these new colonies with the old *h*+ colonies yielded the ratio of the two types of organisms in the culture.¹⁷ Independent determinations of the numbers of organisms in the original cultures of *h*− and *h*+ indicated that the mixture should contain about 48 per cent *h*− bacteria. Direct determinations on the mixture showed that 58 per cent were present. After the mixture had grown to completion for 24 hours 44 per cent *h*− bacteria were present. No appreciable selection of either type had taken place even though growth had occurred through about 11 generations. A second experiment was performed in which the mixture was plated before and after growth directly into minimal medium with 2 per cent washed agar and a limiting concentration of histidine (3 γ per ml.). The *h*+ organisms grew to normal-sized colonies which were easily distinguished from the very small colonies formed by the limited growth of the *h*− bacteria. Comparisons of the numbers of large and small colonies showed that the proportion of *h*− organisms in the mixture before growth was 82 per cent, and after growth for 24 hours, 81 per cent. Once again

there was no evidence of selection for or against the growth of either type of organism in the presence of optimal amounts of histidine.

In the absence of histidine, however, there is a strong selection in favor of *h*+ organisms. When an *h*- culture is washed and 0.05 ml. is inoculated into medium containing optimal amounts of histidine (5-100 γ per ml.) growth is complete at 37°C. in less than 10 hours. When the same inoculum is introduced into minimal medium devoid of histidine there is no growth at 10 hours. But after about 18 hours growth does occur. This growth consists (as determined by plating) almost entirely of *h*+ bacteria which arose from the few *h*+ organisms which were present in the *h*- culture as spontaneous mutants. In the absence of histidine they have been selected for and overgrow the culture. This is clearly shown in an experiment in which 0.05 ml. of mixtures of different proportions of washed *h*- and *h*+ bacteria were inoculated into 10 ml. of medium with and without histidine. The composition of the inoculum and the resulting growth was determined by the first method described above. As can be seen in table 2

TABLE 2
THE EFFECT OF COMPOSITION OF THE INOCULUM ON THE PROPORTION OF HISTIDINELESS BACTERIA IN CULTURES THAT HAVE CEASED TO GROW

PER CENT <i>h</i> - BACTERIA IN INOCULUM	PER CENT <i>h</i> - BACTERIA AFTER GROWTH FOR 24 HOURS ON (γ HISTIDINE PER ML.)		
	25.0	0.4	0
99.1	98.9	23.0	2.1
87.8	78.4	5.3	2.7
54.5	43.9	3.7	1.5
49.6	31.9	2.1	0.5
6.9	9.1	1.6	<0.1

the growth that occurs in the presence of an optimal amount of histidine (25 γ per ml.) does not substantially change the proportion of *h*+ to *h*- bacteria. (This proportion does not shift even after the culture has been in the stationary phase for more than 60 hours.) However, when growth of the mixture occurs in a histidine concentration (0.4 γ per ml.) that is limiting for the *h*- bacteria the *h*+ organisms are selected for and the per cent *h*- bacteria in the growth culture is less than that of the inoculum. The selective advantage of the *h*+ organisms is even greater when the mixtures are inoculated into medium devoid of histidine. The small fraction of *h*- bacteria which are present after final growth are probably supported by traces of histidine in the minimal medium and by histidine secured from the growing *h*+ bacteria. It may be concluded that the *h*- organisms lose out in competition with *h*+ forms in the absence of histidine, while in the presence of an optimal amount neither type seems to have an advantage.

Population Equilibrium.—Ideally the artificial mixture experiment should consist of the introduction of a single *h*+ bacterium into a very large

number of h^- organisms (*ca.* 10^6 - 10^7) in order to mimic the event of mutation. This, however, is not feasible because the variable number of h^+ bacteria which are added by "random" mutation cannot be distinguished from the progeny of the introduced h^+ bacterium without the use of special markers. Additional evidence, however, that there is no positive selection for h^+ organisms in the presence of optimal histidine comes from the determination of the frequency of h^+ bacteria in cultures grown from serially transferred nutrient agar slants. These determinations, shown in table 3, were made over a period of 3 years for other purposes and are not

TABLE 3
THE EFFECT OF SERIAL TRANSFER AND AGING OF h^- CULTURES GROWN ON AGAR SLANTS ON THE PROPORTION OF h^+ BACTERIA IN LIQUID CULTURES ESTABLISHED FROM THEM

NO. OF SERIAL TRANSFER	AGE OF SLANT (MONTHS)	NO. OF h^+ BACTERIA PER 10^8 ORGANISMS
0	1.0	0.17
0	2.7	0.16
0	3.0	0.16
0	4.0	0.11
8	1.4	0.10
8	2.0	0.45
8	2.1	0.88
8	8.4	0.28
9	1.5	0.42
10	0.1	0.15
11	0.1	0.17
13	1.8	0.21
13	2.4	0.59
13	3.4	0.42
13	6.4	0.35
15	4.7	0.24
15	6.4	0.28

direct determinations of the proportion of h^+ bacteria in the growth on the agar slants. Inocula from these slants, containing about 10^7 bacteria, were in each case introduced into medium containing optimal histidine (25 γ per ml.) and after growth was complete the proportion of h^+ bacteria was determined by plating. It can be seen that there is no trend in the values so obtained; the variance is like that due to mutation during growth (see table 1). This suggests that the different stock slants did not contain significantly different proportions of h^+ organisms. The serial transfers of one stock culture to another were made about every 2 months, and since each slant was established from about 10^7 bacteria and contained about 8×10^9 bacteria some 10 generations were involved in its growth. There is no evidence for a progressive selection in favor of the h^+ bacteria during the 150

generations which ensued in the course of the 15 transfers. Indeed, direct platings of a 4.2-month-old 16th transfer and of a 2-day-old 17th transfer showed that they contained 0.18 and 0.15 *h+* bacteria per 10^6 bacteria. These ratios are essentially the same as the values in table 3 and indicate that the proportion does not appreciably change on a slant and that it does not change from slant to slant after serial transfers.

Apparently an equilibrium proportion had been reached in the serially transferred stock slants. Such an equilibrium could be achieved by the balance of forward and backward mutations, selection pressure or both. An analysis of the assumption of this equilibrium will be presented in another communication. For the present it may securely be stated that in the presence of optimal concentrations of histidine there is no selection in favor of *h+* bacteria.

Discussion.—The spontaneous nature of the mutation from *h-* to *h+* is further evidence that the nutritional characteristics of bacteria are controlled by genes which have mutability properties similar to those of sexual organisms, like the mold *Neurospora*.¹⁶ Just as in organisms where mutants induced in the laboratory by x-rays or ultra-violet radiation are similar to those occurring spontaneously and found in nature, so naturally occurring strains of bacteria, like *L. arabinosus* probably have their nutritional requirements controlled by mutable genes. The Lindegrens¹⁸ have found that naturally occurring yeasts have nutritional requirements that are due to single mutable genes. Such growth-factor deficiencies are probably due to spontaneous mutations and, similarly, the development of the ability to dispense with previously required growth factors must, in many cases, be due to spontaneous mutation.

Some nutritional requirements seem more stable than others. This may be due to a favorable selection phenomenon, to an inherent stability of the gene concerned, to the presence or absence of other mutator genes, or to the fact that the synthesis of the substance required is blocked at different steps by mutations in several genes. In the last case the chance that back mutations would occur simultaneously in two or more genes becomes extremely small. Advantage of this fact has been taken in developing a strain of *Neurospora* whose leucine synthesis is blocked at two steps by two different mutant genes. Although when each gene is present by itself in a separate strain adaptation is not infrequent, the double mutant has not been known to adapt in our laboratory. In bacteria, where adaptation to drug resistance has been shown to depend upon gene mutation in some instances,⁹ the simultaneous development of resistance to several different drugs is known to occur so rarely as to be negligible.¹⁹

Although the rate of mutation is critical in determining the stability of a nutritional requirement, selection may exaggerate its influence in an explosive way. In the presence of a limiting amount of growth factor a

culture may be rapidly overgrown by a spontaneously occurring growth factor-independent mutant. For example, by continuously subculturing in the presence of suboptimal concentrations of tryptophane we, like Wright and Skeggs,³ have obtained tryptophane-independent mutants of *L. arabinosus*. Yet these mutants never overgrow cultures maintained in the presence of optimal concentrations of tryptophane. This is a common situation. In the presence of an optimal concentration of growth factor there may be no selection for or against a deficient type. However, selection is not always unimportant in the establishment of deficient mutant types in a population. For example, the adaptation of a mutant of *Neurospora*, which is used for the determination of leucine, has been shown to be due to back mutation of the leucineless gene to a condition where it enables the synthesis of leucine. Although the leucine-independent mutant has a selective advantage in the absence of leucine, in the presence of leucine the leucine-dependent mutant has a selective advantage.¹⁶ This competition is not due to a simple difference in growth rates although this may sometimes be the case^{11, 20}. Either of these mechanisms may have been responsible for the phenomenon found in *Bacterium typhosum*.²¹ A strain of this organism was originally able to utilize ammonia as the sole source of nitrogen. However, after serial transfer on nutrient agar (which contains tryptophane) this ability was lost. An examination of the original ammonia-utilizing culture showed that some organisms were already present which required nitrogen in organic form. It was subsequently shown that strains of *B. typhosum* unable to utilize ammonia required tryptophane.²² There had probably been a selection of tryptophane-dependent bacteria on nutrient agar in the course of the "training."

We may conclude, then, that there are two main factors which must be controlled to keep a culture stable—mutation and selection. The usual methods employed in maintaining stock cultures of bacteria are in most instances manifestly satisfactory. The retention of cultures in the stationary phase of growth in the cold is certainly not conducive to increased mutation. The more growth that is allowed the greater the chance that a mutation will occur. In view of this it may sometimes be wise to grow no more bacteria in stock tubes or in inocula cultures than are needed. For the same reason lyophilized stock cultures are advantageous when the process itself is known to have no influence on mutation or selection. It may also sometimes be helpful to avoid taking samples for inocula directly from the surface of a slant for mutants, when they appear, will be concentrated in the region of their formation. A small sample of a suspension of the contents of a slant surface will have a lesser chance of containing a large number of mutants. In addition, a strain of bacteria with a nutritional requirement ought to be periodically purified by plating and colony isolation.

More important, perhaps, than methods for decreasing mutation are

methods for preventing the selection of mutants once they occur. This can best be achieved by being certain that an optimum level of the required growth factor is used in making medium for stock cultures. The use of "complete" media containing mixtures of natural products may not always be satisfactory for this purpose even though the medium is known to contain an optimum amount of the growth factor. For example, arginine is known to specifically inhibit a lysineless mutant of *Neurospora*.²³ Hence it may be advisable in some cases to use for stock tubes chemically defined medium supplemented with known amounts of growth factor. In most cases the growth factor-dependent organisms will either not be selected against or will have a selective advantage under such conditions. This is probably the mechanism which operates in nature to maintain nutritionally deficient strains.

Summary.—Mutation and selection have been shown to account for the instability of a histidineless strain of *E. coli*. The loss of the requirement for histidine is not induced by the absence of histidine but occurs during the growth of a culture in its presence. Histidine-independent mutants are selected for in the absence (or in the presence of limiting concentrations) of histidine but not in the presence of optimal concentrations. Similar situations are apparently widespread and the maintenance of a stable nutritionally deficient strain of bacteria must take these factors into consideration.

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¹⁴ It should be noted that these data were secured by the plating of undiluted *h*- cultures. When such cultures are diluted and plated the *h*- bacteria form microcolonies which are not visible to the naked eye. Mutations to the *h*+ condition occur in these microcolonies and in some cases this results in the formation of visible *h*+ colonies. These plate mutations obscure the characterization of the number of *h*+ organisms originally present in the *h*- culture. They can be eliminated by omitting asparagine from the minimal medium. Asparagine was present in the medium used for the experiments reported in this paper. Although some plate mutations occur under the crowded conditions formed by plating undiluted cultures, their number is insignificantly small. Even after 5 days less than 5 per cent of all *h*+ colonies are formed by plate mutation, a source of error well within that attributable to random sampling (Ryan, F. J., and Schneider, L. K., *Genetics*, in press). It is interesting to note that a variance analysis of the number of *h*+ colonies on plates of 10^{-6} dilutions of different *h*- cultures indicates that they form a homogeneous population ($P = 0.54$; P for the variance in the number of *h*+ colonies on plates of different samples of the same culture = 0.51). This is independent evidence that 10^{-6} dilutions of *h*- cultures contain no *h*+ organisms but that *h*+ colonies formed on plates of such dilutions are derived from events occurring on the plates.

¹⁵ The spontaneous mutations may conceivably be to a condition of susceptibility to respond to the testing conditions. This notion is operationally the same as mutation to the ability the testing conditions demonstrate.

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A METHOD FOR SELECTION OF BIOCHEMICAL MUTANTS OF *NEUROSPORA**

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Numerous investigations in recent years have firmly established the value of mutants of the mold *Neurospora* as tools for research in biochemistry and genetics.^{1, 5, 6} Although various means have been used for inducing mutations,^{4, 5, 6} the technique of isolation as described by Beadle and

Tatum⁴ has been used by most investigators. This method involves isolation of single ascospores, one from each perithecium, derived from a cross of which one parent was treated. By this method at least 97 per cent of the cultures obtained from the single spore isolations are unchanged wild type strains. In order to obtain specific mutants or a diversity of mutants, it is therefore necessary to isolate a very large number of single ascospores.

The present investigations were undertaken for the purpose of developing a method for obtaining desired mutants with less effort than that required by the previous method of single ascospore isolations.

It was obviously desirable to develop a technique for eliminating a large proportion of wild type strains from those isolated and tested. Such a technique has been developed by Fries, for *Ophiostoma*.⁷ An attempt was made to apply a modified form of the Fries technique to *Neurospora* by removing non-mutated strains by continuous filtration of a suspension of irradiated microconidia that were allowed to germinate and grow on a medium that would support growth of wild type strains but not mutant strains. This procedure was not a success due to a rapid loss in viability of the microconidia used, and because of clogging of the filter by growing mycelia. The few mutants that were obtained by this method occurred as heterocarcinogenic mixtures with wild types.

A successful method has been developed, based on a visual selection of mutants among ascospores germinating on a minimal medium that will support wild type growth but not the growth of biochemical mutants. By this procedure about 80 to 95 per cent of the wild type spores can be discarded without testing.

A major consideration in developing a method of selecting specific mutants is concerned with the effect of a single mutation on the total metabolic pattern of the new strain. There is now ample evidence that the growth of some mutants is inhibited by metabolites which do not affect wild type strains. An outstanding example that has been described in detail is that of the large group of lysineless mutants of *Neurospora*.⁸ These mutants are all inhibited by arginine. It was therefore thought desirable to make the isolation on minimal medium supplemented only with the metabolite that the desired mutant requires for growth. The new method of obtaining mutants by isolating germinating ascospores has been subjected to three types of experiments in order to provide evidence as to its practicability.

1. Germinating mutant ascospores were picked from crosses between known mutants and wild type.

2. A sample of miscellaneous mutant strains (i.e., requiring an amino acid, vitamin, purine or pyrimidine) was picked from a cross of irradiated wild type and untreated wild type.

3. Using appropriate media, specific types of mutants were picked from a cross of irradiated wild type and untreated wild type.

Experimental.—Media: In the experimental work to be described, all crosses were made on the synthetic medium described by Westergaard and Mitchell.⁹ This medium supports the formation of a much larger number of perithecia than does corn meal agar.

The "minimal" medium utilized has been described by Beadle and Tatum.⁴ The "complete" medium, however, has been modified using *Neurospora* extract rather than yeast extract and malt extract. This was prepared from *Neurospora* strain, Abbott 4A, grown with aeration for 3.5 days in 15 liters of minimal medium. The mycelium was collected, chopped and suspended in 4 parts of water. After 20 hours' autolysis under toluene at 37°C., the suspension was autoclaved and filtered. Hydrolyzed casein was added to the filtrate to a concentration of 0.5 mg. %. This preparation was used either as a liquid or solidified with 1.5% agar.

Selection Method: Petri plates (100 mm.) containing 25 ml. of agar medium⁹ were inoculated by spreading a suspension of conidia of one mating type of *Neurospora* over the surface. Following formation of protoperithecia (4 to 6 days) crosses were made by adding a suspension of conidia of the opposite mating type. In cases where irradiation was carried out, the conidia of the second mating type were obtained from a six-day-old culture of wild type grown on minimal medium. These spores were suspended in sterile distilled water and exposed for 4 minutes at a distance of 3.5 in., to radiation from an "Americanaire" ultra-violet lamp (American Sterilizer Co.). More than 95 per cent of the conidia were killed by the treatment. The rayed conidia from one test tube culture were spread over two plates of protoperithecia, producing on incubation, from 1000 to 2000 perithecia. After about 11 days spores were emitted spontaneously and samples were collected on plates containing 25 ml. of medium solidified with 4 per cent agar. Collections were made by inverting the plate containing perithecia over the second plate and thereby allowing the spores to fall on the agar surface. Examinations under the microscope were made at intervals and the collection stopped when the spores were, roughly, 1 mm. apart. The time required to obtain such a distribution varied from 5 to 30 minutes. The plate was then covered and placed at once in an oven at 60°C. for 30 minutes in order to activate the ascospores. This treatment serves also to kill conidia which are usually present. After 12 hours' incubation at 25°C., the wild type ascospores had produced mycelia covering an area about 1-2 mm. in diameter while the mutants were of the order of one-tenth to one-twentieth the size of wild type. Since each perithecium sheds spores into a localized region, the presence of clusters of small forms facilitated detection of mutants. Only one mutant ascospore was taken from a cluster to avoid excessive duplication. The presumed mutant ascospores were picked up on small blocks of agar by use of fine pointed platinum-iridium spatula. They were then placed in a small test tube con-

taining the desired medium. The cultures obtained in this manner were then tested and the mutants identified in much the same manner as that described by Beadle and Tatum.⁴

Selection of Known Mutants: In order to test the method of visual selection of mutants from germinating ascospores, individual crosses were made between wild type and four different known mutants. Ascospores from the perithecia of these crosses were collected on plates of minimal agar and an attempt was made to pick the mutant types. Since in these plates the ratio of mutants to wild type strains was approximately one to one, the task was much simpler than it would be using rayed material where the incidence of mutants is of the order of 2 per cent. Data from this experiment are given in table 1.

TABLE 1
THE VISUAL SELECTION OF MUTANTS FROM CROSSES BETWEEN KNOWN MUTANT AND WILD TYPE STRAINS

MUTANT REQUIREMENT	NO. PICKED	CULTURES OBTAINED	VIABLE SPORES (%)	NO. OF MUTANTS	MUTANTS (%)
Patothenic Acid	45	16	36	16	100
Uridine	55	45	82	45	100
Adenine	55	39	71	38	97
Lysine	45	20	44	18	90

The data of table 1 demonstrate that the method of visual selection of mutants is basically sound.

General Selection of Mutants: A cross was prepared in four plates, using protoperithecia of *Neurospora crassa* 6a and irradiated conidia of 3A. These strains were newly reisolated from a cross of Em5256A and Em 5297a. Spores were shed 11 days after the cross was made and collections on plates of minimal agar were begun at this time. These collections were made each day for 7 days with one final sample taken on the 14th day. The shedding of spores was nearly complete at this time. Approximately 20 germinated spores were picked from each plate at each collection and transferred to tubes of casein, *Neurospora* extract, agar medium. All of the resulting cultures were tested on minimal medium and those that grew in 3 days were discarded. The remaining cultures were tested on the classification mixtures previously described:⁴ (1) vitamins; (2) amino acids; (3) yeast extract and (4) minimal. An additional mixture was also introduced to facilitate classification of the more common types of mutants previously obtained. This mixture contained methionine, 2.5 mg. %; adenine, 5 mg %; cytidine, 3 mg. %; lysine, 10 mg. %; tryptophane, 5 mg. %; and succinic acid, 10 mg. %.

Four kinds of mutants were obtained; those which did not grow on minimal medium but grew when supplied yeast extract or specific known compounds; those which grew very slowly on minimal and complete

media; those which covered the surface of the agar in the tubes but grew no further regardless of the supplement given; and those which were obviously morphological (mostly colonial) mutants. Mutants of the last two categories are not reported here. In addition, mutants collected from the same plate on the same day and which required the same known compound were considered to be duplicates. Only one representative was tabulated. Actually very few cases of such duplications were found.

The results of this experiment are summarized in table 2. Two mutant types are recorded: slow growing mutants and biochemical mutants. The requirements of the majority of the biochemical mutants are also given. In many cases these mutants grew only on the yeast extract classification mixture and their specific requirements were not determined. These are listed as unknown mutants.

TABLE 2
SELECTION OF SLOW GROWING AND BIOCHEMICAL MUTANTS

PLATE NO.	NO. PICKED	CULTURES OBTAINED	NO. SLOW MUTANTS	NO. BIOCHEMICAL MUTANTS	TYPES OF BIOCHEMICAL MUTANTS
1	112	71	3	6	Threonine, arginine methionine, cytidine, unknown (2)
2	163	61	9	8	Proline, cytidine, <i>p</i> -aminobenzoic acid (2), unknown (4)
3	114	62	3	8	Cytidine, methionine, arginine, adenine (2), unknown (3)
4	123	55	12	12	Lysine, methionine, adenine + methionine, tryptophane (2), unknown (2)
Total	512	249	27	34	

It may be observed from the data in table 2 that a wide variety of mutants was obtained from the four plates. Biochemical mutants which did not grow on minimal medium but did grow in the presence of an appropriate supplement constituted 13 per cent of the cultures that were obtained.

Selection of Specific Mutants: Several experiments were designed to select specific mutants from 20 plates prepared as described in the previous section. The first of these was concerned with selection of mutants requiring histidine. No histidineless mutants have been obtained previously in *Neurospora*. Ascospores were collected on minimal medium and, after germination, probable mutants were transferred to minimal medium containing 5 mg. % histidine monohydrochloride. Resulting cultures, 47 from 115 spores picked, were tested on minimal. One failed to grow and it was subsequently established to be a histidineless mutant. This mutant fails to grow in the presence of hydrolyzed casein, yeast extract or *Neurospora* ex-

tract in the concentrations usually used in complete medium. It seems probable that mutants of this type occurred but were eliminated in previous tests because of their failure to grow on the complete medium used.

In a second experiment an attempt was made to select for three types of mutants simultaneously. These were: a frequently occurring type requiring adenine; a type that has occurred only infrequently, requiring pantothenic acid; and a specific mutant, not previously found, that cannot condense indole and serine to form tryptophane. Mutants of the third type might be expected to lack the enzyme which links serine to indole. The presence of this enzyme in wild type *Neurospora* has been reported by Umbreit, *et al.*¹⁰ In order to minimize the selection of other mutants the ascospores were collected on plates of minimal agar supplemented with the following metabolites: charcoal treated, acid hydrolyzed casein, 100 mg. %; riboflavin, 0.5 mg. %; thiamin, 0.25 mg. %; pyridoxine, 0.25 mg. %; cytidine, 3.75 mg. %; indole 5.0 mg. %. These substances were added to minimize the picking of undesired mutant strains. Presumably mutants having these metabolites as growth requirements would be indistinguishable from wild type strains on the plates.

Of 381 ascospores picked from these plates, 265 grew when placed on a medium of minimal agar supplemented with 5 mg. % adenine, 5 mg. % tryptophane and 0.5 mg. % pantothenic acid. On testing the cultures that grew in this medium, it was found that there were no adenine or pantothenic acid mutants but that one tryptophane mutant had been selected. This mutant required tryptophane for growth but did not grow when supplied indole. It was consequently one of the mutants for which the selection was made. No adenine mutants were found in this experiment in spite of the fact that they have occurred at fairly high frequencies in other experiments. It was subsequently found that indole strongly inhibits the growth of adenine mutants, and it is probably because of this previously unknown fact that no adenineless mutants were obtained. Such an inhibition is not surprising in view of the inhibition of growth of bacteria and yeast by the structurally related benzimidazole, investigated by Wooley.¹¹

In a third experiment, ascospores were collected on a medium containing autoclaved fresh liver extract and transferred to tubes containing filter sterilized liver extract. From a total of 145 spores picked, 75 grew when transferred to unheated liver extract medium. One of these cultures failed to grow on minimal medium and has been classified as requiring a heat-labile substance in liver.

Experiments to select for mutants requiring hydrolyzed pantothenic acid or indoleacetic acid were unsuccessful as was an attempt to select a mutant lacking the enzyme urease. In the last case, spores were collected on a medium containing urea as the sole nitrogen source.

Discussion.—It is evident from the experimental data that considerable

progress has been made in developing an improved technique of selecting biochemical mutants in *Neurospora*. Even with the rigorous discarding of duplicate mutants, 13 per cent of the cultures obtained were found to have special growth requirements. Thus, the present method markedly increases the ease with which such mutants can be obtained.

In the selection of specific mutants three were obtained out of seven attempted. The type requiring adenine probably was not obtained because of inhibition by indole. Since the selection experiments were carried out on a small scale, success in all the selections attempted would be unlikely. Thus, 20 Petri dishes containing a total of 20,000 perithecia would have approximately 300 mutants represented, assuming a mutation frequency of 1.5 per cent. Even if all 300 mutants were represented in the ascospores that were picked, the probability of obtaining a particular mutant that occurred infrequently would not be large. By increasing the number of plates of perithecia, the probability of selecting a particular mutant would, of course, be increased.

It has been previously suspected that some types of mutants have been selected against because of the presence of inhibitors in the "complete" medium used for the isolations. Information obtained in this experimental work suggests that such cases are fairly frequent. At least three mutants have now been found that will not grow on the "complete" medium. These require for growth histidine, tryptophane and methionine, respectively. Thus, it is possible that many groups of mutants have not been obtained because of growth inhibition by substances in the medium used for isolations. It is therefore clear that data on the frequency of occurrence of mutants with different growth requirements should be interpreted with this source of error in mind.

Summary.—1. A new method has been developed for isolating biochemical mutants of *Neurospora*.

2. The method has been applied successfully to the selection of some specific mutants not previously obtained. The importance of the use of a simple medium for selecting mutants has been discussed.

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¹ Beadle, G. W., *Ann. Rev. Physiol.*, **10**, 17-42 (1948).

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⁷ Fries, N., *Nature*, **159**, 199 (1947).

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⁹ Westergaard, M., and Mitchell, H. K., *Am. J. Bot.*, **34**, 573-577 (1947).

¹⁰ Umbreit, W. W., Wood, W. A., and Gunsalus, I. C., *J. Biol. Chem.*, **165**, 731-732 (1946).

¹¹ Wooley, D. W., *Ibid.*, **152**, 225-232 (1944).

A PREDICTABLE MUTATION IN BACTERIA

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In the bacterium, *Sarcina lutea*, a color mutation has been found which has a number of interesting characteristics. The mutation is stable and does not revert to the original form either spontaneously or under any treatment thus far attempted. It may be produced at any time by either of two different methods of induction, one environmental, the other chemical. It also occurs spontaneously at a low rate.

If the following four species of bacteria, *Bacillus subtilis*, *Proteus vulgaris*, *B. megatherium* and *S. lutea* are grown together in nutrient broth for twenty-four hours and then reisolated by the dilution method, five different strains instead of four are recovered. In addition to the four original species a new non-pigmented form is always obtained. The new type is similar to *S. lutea* in all morphological features except in its color which is white instead of yellow. This new form is here called *S. alba* and is considered to be a mutant form of *S. lutea*.

The above experiment was repeated 15 times and in every case the same mutant form was obtained. About three times as many yellow colonies as white ones are found when the diluted mixture is plated out. All 15 lines of the white mutant have remained stable for at least 50 transfers at two-day intervals while some of the first to be obtained have been cultured for over a year.

Evidence of the stability of the white mutant is as follows. If *S. lutea* and *S. alba* are grown together in nutrient broth and then plated out both white and yellow colonies are recovered. *S. alba* was inoculated into a medium which consisted of an autoclaved forty-eight-hour nutrient broth culture of *S. lutea*. The *S. alba* grew in this medium without any reversion to the yellow form.

Experiments were performed to determine whether a mixture of all four organisms is necessary to produce the white mutation. Each of the original species was left out in turn and mixtures of three species were tested. A

mixture containing *S. lutea* with *B. megatherium* and *P. vulgaris* did not give rise to the white mutant. Mixtures of *S. lutea* with *B. subtilis* and *P. vulgaris* likewise did not result in any mutations. Mixtures of *S. lutea* with *B. subtilis* and *B. megatherium* gave rise to the white mutation but in a much lower proportion of cases, about sixteen yellow to one white colony.

Mixtures of *S. lutea* with one other species of the original three were made but these mixtures of two organisms did not induce the white mutation in any of the three cases.

Another experiment was made in which *S. lutea* was grown in mixed culture with three entirely different species, *Serratia marcescens*, *B. cereus* and *Corynebacterium xerose*. This mixture of four species did not give rise to any mutations.

Observations on the control cultures showed that *S. lutea* in pure culture undergoes spontaneous mutation to *S. alba* at a rate of about one in 40,000 colonies.

Another white mutation, apparently the same or at least indistinguishable from *S. alba*, can likewise be induced at will by growing *S. lutea* in nutrient broth containing various concentrations of nucleic acid (sodium ribose nucleate). After twenty-four hours of incubation such cultures when plated out yield both white and yellow colonies.

Comparison of these observations with other cases of mutation in bacteria will be made in a future paper. Further genetic tests of this mutation and a physiological comparison of *S. lutea* with *S. alba* are in progress.

A CONVEXITY THEOREM

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The theorem which will be proved here has its origin in the study of the following question.

Let $S_n(x)$ denote the partial sum of order n of the Fourier series of the function $g(x) \in L^p$ ($1 < p \leq 2$). Littlewood and Paley¹ have proved that, $n(x)$ being any measurable function taking non-negative integral values, one has the inequality

$$\int_0^{2\pi} \frac{|S_{n(x)}(x)|^p}{\log [n(x) + 2]} dx \leq A_p \int_0^{2\pi} |g(x)|^p dx, \quad (1)$$

A_p being a constant depending on p only.

The proof of the theorem, when $p \neq 2$,² is extremely long and difficult.

The authors of the present paper have long tried to give a proof which would be based on some theorem analogous to Marcel Riesz's convexity theorem. The difficulty lies on the fact that the above inequality does not hold for $p = 1$. If, however, instead of *Fourier series* of the class L^p , we consider *power series* $f(z) = \sum_0^\infty c_n z^n$ of the Hardy's class H^p , and if we denote by $S_n(\theta)$ the partial sum of order n of the series $f(e^{i\theta}) \sim \sum_0^\infty c_n e^{in\theta}$, then the inequality

$$\int_0^{2\pi} \frac{|S_n(\theta)|^p}{\log [n(\theta) + 2]} d\theta \leq C_p \int_0^{2\pi} |f(e^{i\theta})|^p d\theta \quad (2)$$

holds for $p = 1$ as well as for $p = 2$. For $p = 2$, the result is equivalent to the Littlewood-Paley result. For $p = 1$, the result is due to Zygmund.³ Hence, if one could prove some kind of convexity theorem for functions of the class H^p , the inequality (2) would be proved for $1 \leq p \leq 2$, and (1) would therefore be proved for $1 < p \leq 2$, since it is known that the property H^p for a power series is equivalent to the property L^p for the Fourier series which constitutes its real part, provided that $p > 1$.⁴

The purpose of this note is to give a convexity theorem of this kind. In the proof of it, the authors were inspired by a recent paper of Thorin, and especially by his proof of a theorem of Hardy and Littlewood.⁵ In his proof, as well as in ours, Thorin's own generalization of M. Riesz's convexity theorem plays an important rôle.⁶

THEOREM. Let $f(z) = \sum_0^\infty x_n z^n$ be regular for $|z| < 1$ and belong to the class $H^{1/\alpha}$ ($\alpha > 0$). Let $M(\alpha, \beta)$ denote the maximum of the finite complex bilinear form

$$\sum_{j=0}^m \sum_{h=0}^n a_{jh} x_j y_h$$

when the complex variables x_j, y_h are subject to the conditions

$$\sum_0^n |y_h|^{1/\beta} \leq 1 \quad (\beta \geq 0)^7$$

and

$$\int_0^{2\pi} |f(re^{i\theta})|^{1/\alpha} d\theta \leq 1 \quad (\alpha > 0)$$

the second condition holding for every $f(z)$ belonging to $H^{1/\alpha}$ and having x_0, x_1, \dots, x_m as its $m + 1$ first expansion coefficients, and for every non-negative r inferior to 1. Then, if the point (α, β) lies on the segment joining the points (α_1, β_1) and (α_2, β_2) , that is to say if $\alpha = t\alpha_1 + (1 - t)\alpha_2$ and $\beta = t\beta_1 + (1 - t)\beta_2$ ($0 < t < 1$), we have

$$M(\alpha, \beta) \leq C(\alpha_1, \alpha_2) M^1(\alpha_1, \beta_1) M^{1-\epsilon}(\alpha_2, \beta_2),$$

the constant $C(\alpha_1, \alpha_2)$ depending on α_1 and α_2 only.

Proof. We start from the assumption that the inequality

$$\left| \sum_{j=0}^m \sum_{h=0}^n a_{jh} x_j y_h \right| \leq M(\alpha, \beta) \left[\int_0^{2\pi} |f(e^{i\theta})|^{1/\alpha} d\theta \right]^\alpha \left[\sum_0^n |y_h|^{1/\beta} \right]^\beta \quad (3)$$

holds for the points (α_1, β_1) and (α_2, β_2) , and for all x_j, y_h .

Let κ be the smallest integer such that $\frac{\kappa}{\alpha_1} \geq 2$ and $\frac{\kappa}{\alpha_2} \geq 2$, and let $G(z) = \xi_0 + \xi_1 z + \dots + \xi_p z^p$ be a polynomial of degree p . We now apply the inequality (3) to the case in which $f(z) = G^*(z)$.⁸ Then x_j , that is to say the coefficient of z^j in $f(z)$, becomes a function $\varphi_j(\xi_0, \xi_1, \dots, \xi_p)$, which is a polynomial, homogeneous, of degree κ , whose coefficients depend on κ and p only. Then (3) becomes

$$\left| \sum_{j=0}^m \sum_{h=0}^n a_{jh} \varphi_j(\xi_0, \dots, \xi_p) y_h \right| \leq M(\alpha, \beta) \left[\int_0^{2\pi} |G(e^{i\theta})|^{\kappa/\alpha} d\theta \right]^\alpha \left[\sum_0^n |y_h|^{1/\beta} \right]^\beta \quad (4)$$

and is satisfied for the points (α_1, β_1) and (α_2, β_2) , and for all values of ξ_0, \dots, ξ_p and y_0, \dots, y_n .

Now, by a known theorem on interpolation⁹ there exist, for all exponents $q \geq 2$, well-determined constants $A_{(q)}$ and $B_{(q)}$ such that

$$A_{(q)} \frac{|G_0|^q + |G_1|^q + \dots + |G_p|^q}{p+1} \leq \int_0^{2\pi} |G(e^{i\theta})|^q d\theta \leq B_{(q)} \frac{|G_0|^q + \dots + |G_p|^q}{p+1},$$

where $G_s = G(e^{i\theta_s})$ and $\theta_s = \frac{2\pi}{p+1} s$.

We now change variables, taking, instead of ξ_0, \dots, ξ_p , the variables

$$\eta_s = \xi_0 + \xi_1 e^{i\theta_s} + \dots + \xi_p e^{i p \theta_s} = G_s$$

as is clearly possible, the determinant being different from zero. The polynomial $\varphi_j(\xi_0, \dots, \xi_p)$ becomes a polynomial $\psi_j(\eta_0, \dots, \eta_p)$, and we get,

writing $B = \max. \left\{ B\left(\frac{\kappa}{\alpha_1}\right), B\left(\frac{\kappa}{\alpha_2}\right) \right\}$

$$\left| \sum_{j=0}^m \sum_{h=0}^n a_{jh} \psi_j(\eta_0, \dots, \eta_p) y_h \right| \leq M(\alpha, \beta) \left[B \frac{|\eta_0|^{\kappa/\alpha} + \dots + |\eta_p|^{\kappa/\alpha}}{p+1} \right]^\alpha \left[\sum_0^n |y_h|^{1/\beta} \right]^\beta$$

this inequality being valid for the points (α_1, β_1) and (α_2, β_2) and for all η_0, \dots, η_p and y_0, \dots, y_n .

We apply now Thorin's generalization of Marcel Riesz's convexity theorem, and we get, writing A instead of $A\left(\frac{\kappa}{\alpha}\right)$,

$$\begin{aligned} \left| \sum_{j=0}^m \sum_{h=0}^n a_{jh} \psi_j(\eta_0 \dots \eta_p) y_h \right| &\leq \\ &\left[M(\alpha_1, \beta_1) \frac{B^{\alpha_1}}{(p+1)^{\alpha_1}} \right]^t \left[M(\alpha_2, \beta_2) \frac{B^{\alpha_2}}{(p+1)^{\alpha_2}} \right]^{1-t} \left[\sum_0^p |\eta_s|^{\kappa/\alpha} \right]^\alpha \left[\sum_0^n |y_h|^{1/\beta} \right]^\beta \leq \\ &M^t(\alpha_1, \beta_1) \cdot M^{1-t}(\alpha_2, \beta_2) \left[\frac{B}{p+1} \sum_0^p |\eta_s|^{\kappa/\alpha} \right]^\alpha \left[\sum_0^n |y_h|^{1/\beta} \right]^\beta \leq \\ &\left(\frac{B}{A} \right)^\alpha M^t(\alpha_1, \beta_1) M^{1-t}(\alpha_2, \beta_2) \left[\int_0^{2\pi} |G(e^{i\theta})|^{\kappa/\alpha} d\theta \right]^\alpha \left[\sum_0^n |y_h|^{1/\beta} \right]^\beta \end{aligned}$$

Thus also

$$\begin{aligned} \left| \sum_{j=0}^m \sum_{h=0}^n a_{jh} x_j y_h \right| &\leq \\ &\left(\frac{B}{A} \right)^\alpha M^t(\alpha_1, \beta_1) M^{1-t}(\alpha_2, \beta_2) \left[\int_0^{2\pi} |f(e^{i\theta})|^{1/\alpha} d\theta \right]^\alpha \left[\sum_0^n |y_h|^{1/\beta} \right]^\beta \end{aligned}$$

where x_0, x_1, \dots, x_m are the first $m+1$ coefficients of $f(z) = G^*(z)$, $G(z)$ being a polynomial and κ being fixed as said before.

The last inequality being true when G is any polynomial, it remains true when G is any function of the class $H^{\kappa/\alpha}$. Hence it is also true when $f(z)$ is any function of the class $H^{1/\alpha}$ and having no zeros for $|z| < 1$, for then we can always write $f(z) = G^*(z)$, G being regular.

From this we deduce, since any function $f(z)$ of the class $H^{1/\alpha}$ is the sum of two "zero-free" functions $f_1(z)$ and $f_2(z)$ of the same class, with $|f_1| \leq 2|f|$ and $|f_2| \leq 2|f|$, that, D being an absolute constant,

$$\begin{aligned} \left| \sum_{j=0}^m \sum_{h=0}^n a_{jh} x_j y_h \right| &\leq \\ &\left(\frac{B}{A} \right)^\alpha D M^t(\alpha_1, \beta_1) M^{1-t}(\alpha_2, \beta_2) \left[\int_0^{2\pi} |f(e^{i\theta})|^{1/\alpha} d\theta \right]^\alpha \left[\sum_0^n |y_h|^{1/\beta} \right]^\beta \end{aligned}$$

for any function $f \in H^{1/\alpha}$ regular for $|z| < 1$. This gives just our theorem, if we observe that $\alpha_1 < \alpha < \alpha_2$, that κ and B depend only on α_1 and α_2 , and that the minimum of $A = A\left(\frac{\kappa}{\alpha}\right)$, when $\frac{\kappa}{\alpha}$ lies between $\frac{\kappa}{\alpha_1}$ and

$\frac{\kappa}{\alpha_2}$, depends also on α_1 and α_2 only.

Writing $X_h = \sum_{j=0}^{\infty} a_{jh} x_j$ ($h = 0, 1, \dots, n$), it is familiar that $M(\alpha, \beta)$ is also the maximum of the ratio $(\sum_0^n |X_h|^{1/\beta'})^{\beta'}/[\int_0^{2\pi} |f(e^{i\theta})|^{1/\alpha}]^\alpha$, where $\beta + \beta' = 1$. From sums of powers of linear forms we pass, by the classical process, to integrals, and the theorem of Littlewood and Paley is the consequence of the inequality (2) applied to the two extreme cases $\alpha = \beta' = 1/2$ ($p = 2$) and $\alpha = \beta' = 1$ ($p = 1$).

Remark: In our main theorem, the condition on the y 's can be replaced by one similar to that imposed on the x 's. But this has no interesting application. By passages to limits we can also obtain results about linear operations from H^p to L^q , where $p, q \geq 1$.

¹ Littlewood and Paley, *Proc. London Math. Soc.*, **43**, 105 (1937).

² For a simple proof in the case of $p = 2$, see Hardy and Littlewood, *Proc. Cambridge Phil. Soc.*, **40**, 103-107 (1938).

³ See Zygmund, A., *Fundamenta Mathematicae*, **30**, 190 (1938).

⁴ A function $f(z) = \sum c_n z^n$, regular for $|z| < 1$, is said to belong to the class H^p if $\int_0^{2\pi} |f(re^{i\theta})|^p d\theta$ remains bounded as $r \rightarrow 1$. For the classical results of the theory see, e.g., Zygmund, *Trigonometrical Series*, Chapter VII.

⁵ Thorin, G. O., *Convexity Theorems*, Uppsala, 1948, pp. 1-57, esp. 31-35.

⁶ Thorin, G. O., "An Extension of a Convexity Theorem Due to M. Riesz," *Kungl. Fysiografiska Sällskapet i Lund Förhandlingar*, **8** (1939), nr. 14. Tamarkin, J. D., and Zygmund, A., "Proof of a Theorem of Thorin," *Bull. the Am. Math. Soc.*, **50**, 279-282 (1944). Salem, R., Sur une extension du théorème de convexité de M. Marcel Riesz, *Colloquium Mathematicum*, Wrocław, 1947, Vol. 1, pp. 6-8.

⁷ In the case $\beta = 0$, the condition is to be interpreted, as usual, as $\max |y_h| \leq 1$.

⁸ Thorin's idea (see footnote 5) of taking for f the k th power of an analytic function and applying his convexity theorem, is basic for the whole argument. If we wanted to restrict ourselves to the proof of the Littlewood-Paley result, the argument could be simplified still further, since here we interpolate between $\alpha = 1$ and $\alpha = 1/2$, and we could imitate more closely the proof of Thorin (see footnote 5, pp. 31-35).

⁹ See e.g. Marcinkiewicz and Zygmund, *Fundamenta Mathematicae*, **28**, 131-166 (1937).

NOTES ON INTEGRATION, II

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Communicated July 6, 1946

The results of our first note¹ enable us to treat the following classes of functions, all contained in \mathfrak{G} :

$$\begin{aligned} \mathfrak{B}_p &= \{f; f|f|^{p-1} \in \mathfrak{F}\} = \{f; N(|f|^p) < +\infty\}, & \mathfrak{B}_p &\subset \{f; f|f|^{p-1} \in \mathfrak{L}\}, \\ \mathfrak{M} &= \{f; \text{mid}(f, g, h) \in \mathfrak{L} \text{ for all } g \text{ and } h \text{ in } \mathfrak{L}\}, \end{aligned}$$

where $p \geq 1$ and $\text{mid}(\lambda, \mu, \nu)$ designates the intermediate one of the three numbers λ, μ, ν in accordance with the precise relations

$$\begin{aligned}\text{mid}(\lambda, \mu, \nu) &= \max(\min(\lambda, \mu), \min(\mu, \nu), \min(\nu, \lambda)) \\ &= \min(\max(\lambda, \mu), \max(\mu, \nu), \max(\nu, \lambda)).\end{aligned}$$

Obviously, $\mathfrak{F}_1 = \mathfrak{F}$ and $\mathfrak{L}_1 = \mathfrak{L}$. The importance of the class \mathfrak{L}_p is well established, and the consideration of \mathfrak{F}_p along with \mathfrak{L}_p is natural. Since in the classical instances of our theory \mathfrak{M} can be identified as the totality of measurable functions, we shall call any function in \mathfrak{M} a *measurable function*.

In order to discuss \mathfrak{F}_p we need to establish for the quantity $N_p(f) = N(|f|^p)^{1/p}$ the inequalities

- (1) (Hölder) if $p > 1$ and $p + q = pq$, then $N(fg) \leq N_p(f)N_q(g)$;
- (2) (Minkowski) $N_p(f + g) \leq N_p(f) + N_p(g)$.

The proof of (1) begins with the observation that for $\alpha > 0$, $\beta > 0$, and $\gamma = 1/[(q/p)^{1/q} + (p/q)^{1/p}]$ the function $\gamma(\alpha\xi^p + \beta\xi^{-q})$, $0 < \xi < +\infty$, has $\alpha^{1/p}\beta^{1/q}$ as its absolute minimum and assumes this value only when $\xi = \xi_0 = (\beta q/\alpha p)^{1/pq}$. As a result we see that $|fg| \leq \gamma(|f|^p\xi^p + |g|^q\xi^{-q})$ and hence that $N(fg) \leq \gamma(N(|f|^p)\xi^p + N(|g|^q)\xi^{-q})$ for all $\xi > 0$. On putting $N(|f|^p) = \alpha$, $N(|g|^q) = \beta$, and $\xi = \xi_0$ in the latter inequality we obtain (1). Since for $p = 1$ the inequality (2) has already been established (as a special case of I (7)), we suppose that $p > 1$. The proof for this case then begins with the observation that $|\xi + \eta|^p \leq 2^{p-1}(|\xi|^p + |\eta|^p)$. We therefore have $N(|f + g|^p) \leq 2^{p-1}(N(|f|^p) + N(|g|^p))$, so that $f + g$ is in \mathfrak{F}_p whenever f and g are. Consequently we can use (1) and the relation $|f + g|^p \leq |f||f + g|^{p-1} + |g||f + g|^{p-1}$ to obtain

$$\begin{aligned}N(|f + g|^p) &\leq N(|f||f + g|^{p-1}) + N(|g||f + g|^{p-1}) \\ &\leq (N_p(f) + N_p(g))N(|f + g|^p)^{1/q}\end{aligned}$$

an inequality from which (2) follows at once. The Minkowski inequality shows immediately that identification of functions f and g for which $N_p(f - g) = 0$ will permit us to treat \mathfrak{F}_p as a real normed vector-lattice with N_p as its norm-function. It is easily verified that this identification is the same for all values of p , two functions being identified if and only if they are equal almost everywhere. We can now generalize I (10) to read

- (3) the normed vector space \mathfrak{F}_p is complete (and hence a Banach space).

The proof will be sketched for $p > 1$. By definition the mapping Φ which carries f into $g = |f||f|^{p-1} = |f|^p \text{sgn } f$ maps \mathfrak{F}_p onto \mathfrak{F} . It has as its inverse the mapping Ψ which carries g into $f = |g|^{1/p} \text{sgn } g$. The local

behavior of these mappings can be determined by an appeal to the inequalities²

$$2^{1-p}|\xi - \eta|^p \leq |\xi|\xi|^{p-1} - \eta|\eta|^{p-1}| \leq p|\xi - \eta|(|\xi|^{p-1} + |\eta|^{p-1}),$$

which hold even for complex ξ and η . The first yields $|\Psi(g_1) - \Psi(g_2)|^p \leq 2^{p-1}|g_1 - g_2|^p$ and hence $N_p(\Psi(g_1) - \Psi(g_2)) \leq 2^{1/q}N(g_1 - g_2)^{1/p}$ for all g_1 and g_2 in \mathfrak{F} . Accordingly Ψ is continuous, belonging in fact to the Lipschitz class $\text{Lip } 1/p$. Similarly, the second inequality yields $|\Phi(f_1) - \Phi(f_2)| \leq p(|f_1 - f_2||f_1|^{p-1} + |f_1 - f_2||f_2|^{p-1})$ and hence, with the help of the Hölder inequality,

$$N(\Phi(f_1) - \Phi(f_2)) \leq pN_p(f_1 - f_2)(N_p(f_1)^{p/q} + N_p(f_2)^{p/q}).$$

Accordingly Φ is continuous, belonging on any bounded part of \mathfrak{F}_p to the associated Lipschitz class $\text{Lip } 1$. If now $\{f_n\}$ is a Cauchy sequence in \mathfrak{F}_p , it is bounded and must therefore be carried by Φ into a Cauchy sequence $\{g_n\}$, $g_n = \Phi(f_n)$, in \mathfrak{F} . The completeness of \mathfrak{F} shows that the latter sequence has a limit g in \mathfrak{F} . Thus the function $f = \Psi(g)$ is the limit of $\{f_n\}$ in \mathfrak{F}_p , by virtue of the continuity of Ψ . This completes the proof. We note further that we have obtained at the same time the following variant of a result of Mazur:²

(4) *The spaces \mathfrak{F}_p , $p \geq 1$, are mutually homeomorphic and all have the same linear dimension.*

The first part of (4) follows from the fact that Φ maps \mathfrak{F}_p homeomorphically onto \mathfrak{F} , as shown above. The second results from a topological interpretation of the linear dimension. It is well known that a Banach space has finite linear dimension if and only if it is locally compact, in which case it is homeomorphic to an n -dimensional Euclidean space and its linear dimension is n . On the other hand, if the linear dimension of a Banach space is infinite it is equal to the density-character of the space—that is, to the least among the cardinal numbers of everywhere dense parts of the space.

Turning now to the consideration of \mathfrak{L}_p , we proceed to specialize and sharpen the results of the previous paragraph. First we replace (1) by the more detailed statement

- (1') (Hölder) *if $f \in \mathfrak{L}_p$ and $g \in \mathfrak{L}_q$, then $fg \in \mathfrak{L}$ and $|L(fg)| \leq L(|f|^p)^{1/p} \cdot L(|g|^q)^{1/q}$, the equality holding if and only if $f|f|^{p-1}$ and $g|g|^{q-1}$ are linearly dependent in \mathfrak{L} (when $p = q = 2$, if and only if f and g are linearly dependent in \mathfrak{L}_2).*

To show that $fg \in \mathfrak{L}$ under the present hypotheses, we appeal to I (13), writing $fg = \varphi(f', g')$ where $f' = f|f|^{p-1} \in \mathfrak{L}$, $g' = g|g|^{q-1} \in \mathfrak{L}$, and $\varphi(\lambda, \mu) = |\lambda|^{1/p}|\mu|^{1/q} \operatorname{sgn} \lambda\mu$. Thus we have

$$|L(fg)| \leq L(|fg|) = N(fg) \leq N_p(f)N_q(g) = L(|f|^p)^{1/p}L(|g|^q)^{1/q}$$

by virtue of (1). In determining the conditions under which the extreme terms here are equal, we may discard the trivial case where the last term vanishes, one of the two functions f and g being a null function. The indicated equality is equivalent to the continued equation $\pm L(fg) = L(|fg|) = L(|f|^p)\xi_0^p + L(|g|^q)\xi_0^{-q} = L(|f|^p\xi_0^p + |g|^q\xi_0^{-q})$, where ξ_0 has the value indicated in the proof of (1) and a fixed determination of the ambiguous sign is adopted for the remainder of the discussion. In view of the relations $\pm fg \leq |fg| \leq |f|^p\xi_0^p + |g|^q\xi_0^{-q}$, the continued equation for the integrals holds if and only if $\pm fg = |fg| = |f|^p\xi_0^p + |g|^q\xi_0^{-q}$ almost everywhere. The second equation here holds if and only if $|g|^q = (p/q)\xi_0^q|f|^p$ almost everywhere. Consequently both equations hold if and only if $g|g|^{q-1} = \pm(p/q)\xi_0^q|f|^{p-1}$ almost everywhere. The proof of (1') is thereby completed. In a quite similar fashion we find that (2) can be replaced by

(2') (Minkowski) if f and g are in \mathfrak{L}_p , then $f + g$ is in \mathfrak{L}_p and $L(|f + g|^p)^{1/p} \leq L(|f|^p)^{1/p} + L(|g|^p)^{1/p}$ the equality holding if and only if f and g are linearly dependent in \mathfrak{L}_p and of the same sign almost everywhere.

To show that $f + g \in \mathfrak{L}_p$ under the present hypotheses, we appeal to I (13), writing $(f + g)|f + g|^{p-1} = \varphi(f', g')$ where $f' = f|f|^{p-1} \in \mathfrak{L}$, $g' = g|g|^{p-1} \in \mathfrak{L}$, and $\varphi(\lambda, \mu) = (|\lambda|^{1/p} \operatorname{sgn} \lambda + |\mu|^{1/p} \operatorname{sgn} \mu) \{ |\lambda|^{1/p} \operatorname{sgn} \lambda + |\mu|^{1/p} \operatorname{sgn} \mu \}^{p-1}$. The result given in (2) now assumes the present form. Reviewing the proof of (2) under the conditions postulated here we see that the equality holds if and only if $|f + g| = |f| + |g|$ almost everywhere while $|f|^p$, $|g|^p$, and $|f + g|^p$ are linearly dependent in \mathfrak{L} in harmony with (1'); but these conditions are equivalent to those stated above. Since \mathfrak{L}_p clearly contains $\alpha f'$ and $|f|$ together with f , it is a linear sublattice of \mathfrak{F}_p . Moreover the fact that the homeomorphic mapping Φ carries \mathfrak{L}_p onto \mathfrak{L} , where \mathfrak{L} is closed in \mathfrak{F} , shows that \mathfrak{L}_p is closed in \mathfrak{F}_p . Accordingly we have

(3') \mathfrak{L}_p is a closed linear subspace of \mathfrak{F}_p (and hence is a Banach space).

We can give a rather wide extension of (4) which will cover not only the spaces \mathfrak{L}_p , $p \geq 1$, arising from a fixed elementary integral but also the spaces \mathfrak{L}_p arising from different elementary integrals. This is possible because the space \mathfrak{L}_2 is a generalized Euclidean or real Hilbert space, its norm being obtained in the appropriate manner from the integral $L(fg)$, which depends linearly and symmetrically upon f and g . Two such spaces are homeomorphic if and only if they have the same linear dimension, as is well known. Hence we have

- (4') the spaces \mathfrak{L}_p , $p \geq 1$, arising from a fixed elementary integral are mutually homeomorphic and all have the same linear dimension; two such spaces arising from different elementary integrals are homeomorphic if and only if they have the same linear dimension.

This result completes our discussion of the spaces \mathfrak{L}_p .

In the study³ of the class \mathfrak{M} , it is convenient to observe that $\text{mid}(\lambda, \mu, \nu)$ is a positively homogeneous, continuous function satisfying the inequalities $\min(\mu, \nu) \leq \text{mid}(\lambda, \mu, \nu) \leq \max(\mu, \nu)$, since we thereby justify applications of I (13) and of the dominated-convergence theorem at various points below. It is, of course, true that $\text{mid}(\lambda, \mu, \nu)$ has an explicit expression in terms of λ, μ, ν by means of addition, multiplication by constants and formation of absolute values. Thus we can show at once that

- (5) if $f = \lim_{n \rightarrow \infty} f_n$ and $f_n \in \mathfrak{M}$, then $f \in \mathfrak{M}$.

In fact we have $\text{mid}(f_n, g, h) \in \mathfrak{L}$ whenever g and h are in \mathfrak{L} , and hence conclude by the dominated-convergence theorem that $\text{mid}(f, g, h) = \lim_{n \rightarrow \infty} \text{mid}(f_n, g, h)$ is also in \mathfrak{L} . We also have

- (6) if f_1, \dots, f_m are in \mathfrak{M} and if $\varphi(\lambda_1, \dots, \lambda_m)$ is any positively homogeneous function of Baire (bounded or not) defined for $-\infty \leq \lambda_k \leq +\infty$ then $\varphi(f_1, \dots, f_m) \in \mathfrak{M}$; in particular, the functions $\alpha f, |f|, f + g$ are measurable whenever f and g are.

Once the indicated particular cases have been handled, the general case is obtained by passages to the limit similar to those used in deriving I (13) from I (11). Taking the case of $|f|$ as typical, we wish to show that $\text{mid}(|f|, g, h) \in \mathfrak{L}$ whenever g and h are in \mathfrak{L} . By hypothesis the function $f_n = \text{mid}(f, n(|g| + |h|), -n(|g| + |h|))$ is in \mathfrak{L} ; and so also is the function $g_n = \text{mid}(|f_n|, g, h)$. It is easily seen that $\lim_{n \rightarrow \infty} f_n(x)$ has the value 0 or the value $f(x)$ according as $|g(x)| + |h(x)|$ vanishes or not. Hence $\lim_{n \rightarrow \infty} g_n = \text{mid}(|f|, g, h)$, and the latter function is in \mathfrak{L} by the dominated-convergence theorem. In practice it is convenient to have available the following criterion:

- (7) when $f \geq 0$, a necessary and sufficient condition for f to be measurable is that $\min(f, g) \in \mathfrak{L}$ whenever $g \in \mathfrak{L}$ and $g \geq 0$.

The necessity of the stated condition results immediately from the identity $\min(f, g) = \text{mid}(f, g, 0)$. The sufficiency is established by noting that for arbitrary g and h in \mathfrak{L} we have $\text{mid}(f, g, h) = \max(\min(f, \max(g, h)), \min(g, h)) \in \mathfrak{L}$ since $\min(f, \max(g, h)) = \min(f, \max(g, h))$.

0)) $+\min(0, \max(g, h)) \in \mathfrak{L}$ by hypothesis. From (7) and I (14) we have

- (8) *the constant function 1 is measurable if and only if $\min(1, g)$ is integrable whenever g is; and it is measurable whenever I (3) is verified.*

Hence we immediately obtain the following generalization of (6) above

- (9) *if $1, f_1, \dots, f_m$ are measurable and if $\varphi(\lambda_1, \dots, \lambda_m)$ is any function of Baire (bounded or not) defined for $-\infty \leq \lambda_k \leq +\infty$, then $\varphi(f_1, \dots, f_m) \in \mathfrak{M}$.*

We conclude our general remarks on measurable functions by noting some criteria for integrability. First we have

- (10) *in order that a measurable function f be integrable it is necessary and sufficient that $N(f) < +\infty$; and it is likewise necessary and sufficient there exist integrable functions g and h , such that $g \leq f \leq h$.*

If f is integrable, then $N(f) < +\infty$ and the second condition is satisfied with $g = h = f$. If $N(f) < +\infty$ we can find a function $k \in \mathfrak{L}$ such that

$|f| \leq k$, since there exist elementary functions f_n such that $|f| \leq \sum_{n=1}^{\infty} |f_n|$, $\sum_{n=1}^{\infty} E(|f_n|) < +\infty$, and the function $k = \sum_{n=1}^{\infty} |f_n|$ is in \mathfrak{L} by virtue of I (12).

Thus $N(f) < +\infty$ implies that $g \leq f \leq h$ where $g = -k$ and $h = k$ are in \mathfrak{L} . When there exist such g and h , we have $f = \text{mid}(f, g, h) \in \mathfrak{L}$ by the definition of \mathfrak{M} . Since any integrable function is clearly measurable, by direct application of the definition, we see that (10) yields the relations

$$(11) \quad \mathfrak{L} = \mathfrak{M} \cap \mathfrak{F} = \{f; f \in \mathfrak{M}, N(f) < +\infty\}.$$

It follows immediately that

$$(12) \quad \mathfrak{L}_p = \{f; |f|^{p-1} \in \mathfrak{M}, N_p(f) < +\infty\}.$$

When 1 is measurable we can give the last result the sharper form,

$$(13) \quad \text{if } 1 \in \mathfrak{M}, \text{ then } \mathfrak{L}_p = \mathfrak{M} \cap \mathfrak{F}_p = \{f; f \in \mathfrak{M}, N_p(f) < +\infty\},$$

since (9) shows that the functions $g = |f|^{p-1}$ and $f = |g|^{1/p} \text{sgn } g$ are measurable together.

Finally we shall consider the connections between our theory of the general integral and the theory of measure. By specializing I (5)–(7) we see that an outer measure μ^* is defined for the subsets of X by putting $\mu^*(Y) = N(f_Y)$ where f_Y is the characteristic function of $Y \subset X$. Similarly, we see from the properties of the general integral that the finite set-function μ defined by putting $\mu(Y) = L(f_Y)$ whenever $f_Y \in \mathfrak{L}$ is a *completely additive*

measure. It is natural to define a set Y to be measurable if and only if $f_Y \in \mathfrak{M}$; but we must then consider how such sets are related to the μ^* -measurable sets Y , characterized by the now classical condition that $\mu^*(Z) = \mu^*(Z \cap Y) + \mu^*(Z \cap Y')$ for all Z . A fundamental tool in this investigation is provided by the following result:

- (14) *if $N(f) < +\infty$, there exists a function $g \in \mathfrak{L}$ such that $|f| \leq g$ and $N(f) = L(g)$ —in particular, if $1 \in \mathfrak{M}$ and f is a characteristic function, then g may be chosen to be a characteristic function.*

Adopting a device used in the proof of (10) above, we select elementary functions f_{mn} such that $|f| \leq \sum_{n=1}^m |f_{mn}| = g_m$, $N(f) \leq \sum_{n=1}^m E(|f_{mn}|) \leq N(f) + 1/m$ and note that $g_m \in \mathfrak{L}$ and $N(f) \leq L(g_m) \leq N(f) + 1/m$ in accordance with I (12). It is then clear that $g = \lim_{m \rightarrow \infty} \min(g_1, \dots, g_m)$ has the desired properties. In the indicated special case, we may first suppose that $g \leq 1$ since otherwise we can replace g by $\min(1, g) \in \mathfrak{L}$ in accordance with (8); and we may then suppose that g is a characteristic function, since otherwise we can replace g by the characteristic function $\lim_{n \rightarrow \infty} g^n \in \mathfrak{L}$, in accordance with (9), (10) and the dominated-convergence theorem. From (14) we obtain

- (15) *every measurable set is μ^* -measurable; but the converse is true if and only if $1 \in \mathfrak{M}$.*

We sketch the proof. Assuming Y to be measurable, we have to reduce the inequality $\mu^*(Z) \leq \mu^*(Z \cap Y) + \mu^*(Z \cap Y')$ to an equality. Since the reduction is automatic when $\mu^*(Z) = +\infty$, we suppose that $\mu^*(Z) < +\infty$. Then (14) furnishes a function $g \in \mathfrak{L}$ such that $f_Z \leq g$, $\mu^*(f_Z) = L(g)$. Since $f_{Z \cap Y} = f_Z f_Y \leq g f_Y = \min(g, f_Y) \leq g$ we see that $g f_Y \in \mathfrak{L}$, $\mu^*(Z \cap Y) \leq L(g f_Y)$. The inequality $f_{Z \cap Y'} = f_Z(1 - f_Y) \leq g(1 - f_Y) = g - g f_Y \in \mathfrak{L}$ shows that $\mu^*(Z \cap Y') \leq L(g - g f_Y)$. By addition we obtain $\mu^*(Z \cap Y) + \mu^*(Z \cap Y') \leq L(g f_Y) + L(g - g f_Y) = L(g) = \mu^*(Z)$, thereby completing the discussion. Looking now at the converse, we see that X is trivially μ^* -measurable and hence that the converse cannot hold unless $1 = f_X \in \mathfrak{M}$. Assuming this necessary condition, we now show that $f_Y \in \mathfrak{M}$ by (7), whenever Y is μ^* -measurable. Starting with an arbitrary non-negative integrable function g , we put $f_n = \varphi_n(g) \leq ng, g_n = g f_n \leq g$ where φ_n is the characteristic function of the interval $1/n \leq \lambda \leq +\infty$. By (9) and (10) we see that f_n and g_n are integrable. Moreover f_n is the characteristic function of a set Z_n with $\mu(Z_n) = L(f_n)$. Since $\min(f_Y, g) = \lim_{n \rightarrow \infty} \min(f_Y, g_n) = \lim_{n \rightarrow \infty} (f_Y f_n, g) \leq g$ it suffices to show that $f_Y f_n \in \mathfrak{L}$. Since $f_Y f_n$ is the characteristic function of $Y \cap Z_n$, the special

case of (14) furnishes us with a set W_n such that $Y \cap Z_n \subset W_n$, $\mu^*(Y \cap Z_n) = \mu(W_n)$. We may suppose that $W_n \subset Z_n$, since otherwise we can replace it by $W_n \cap Z_n$. We now have $\mu(W_n) = \mu^*(W_n) = \mu^*(W_n \cap Y) + \mu^*(W_n \cap Y') \geq \mu^*(W_n \cap Y) = \mu^*(Y \cap Z_n) = \mu(W_n)$. Hence $\mu^*(W_n \cap Y') = 0$ and $W_n \cap Y'$ is a null set. Thus $f_Y f_n$ differs from the characteristic function of W_n by a null function and is integrable. This completes the proof. From (14) and (15) we at once derive

- (16) *when $1 \in \mathfrak{M}$, $\mu^*(Y) < +\infty$ is the minimum of the measures $\mu(Z)$ where $Z \supset Y$; and $\mu(Y)$ exists if and only if Y is μ^* -measurable and has finite outer measure, in which case $\mu(Y) = \mu^*(Y)$.*

On the basis of the preceding results, we can now establish

- (17) *when $1 \in \mathfrak{M}$, a finite function f is measurable if and only if the sets $\{x; \alpha \leq f(x) < \beta\}$ are measurable for all (rational) α and β , $\alpha > \beta$; and, when f is integrable, its general integral $L(f)$ is the limit of the Lebesgue sums*

$$\sigma(f; \epsilon) = \sum_{k=-\infty}^{k=+\infty} \sigma_k \mu\{x; \alpha_k \leq f(x) < \alpha_{k+1}\},$$

where $\lim_{k \rightarrow -\infty} \alpha_k = -\infty$, $\lim_{k \rightarrow +\infty} \alpha_k = +\infty$, $\alpha_k \leq \sigma_k \leq \alpha_{k+1}$, $\alpha_{k+1} - \alpha_k \leq \epsilon$, and $\sigma_k = 0$ for $\min(|\alpha_k|, |\alpha_{k+1}|) < \epsilon$, the summation omitting those terms in which $\sigma_k = 0$.

The measurability of the set $\{x; \alpha \leq f(x) < \beta\}$ is equivalent to that of its characteristic function, expressible as $\varphi_{\alpha\beta}(f)$ where $\varphi_{\alpha\beta}$ is the characteristic function of the interval $\alpha \leq \lambda < \beta$. By (9) this function is measurable when f is. On the other hand, if $\varphi_{\alpha\beta}(f)$ is measurable for all α and β

(or even just for rational α and β), we see that the function $f_\epsilon = \sum_{k=-\infty}^{k=+\infty} \sigma_k \varphi_k(f)$, where $\varphi_k = \varphi_{\alpha_k \alpha_{k+1}}$, is measurable (for rational α_k , at least) and that its limit $f = \lim_{\epsilon \rightarrow 0} f_\epsilon$ is also, in accordance with (5) and (6). When f is integrable and $\gamma_k = \min(|\alpha_k|, |\alpha_{k+1}|) \geq \epsilon$, we see that $\varphi_k(f) \leq |f|/\gamma_k$,

$|f_\epsilon| \leq 2 \sum_{k=-\infty}^{k=+\infty} \gamma_k \varphi_k(f) \leq 2|f|$ by virtue of the inequality $|\sigma_k| \leq \min(|\alpha_k|, |\alpha_{k+1}|) + \epsilon \leq 2\gamma_k$; and hence that $\varphi_k(f)$ and f_ϵ are both integrable in accordance with (10). The dominated-convergence theorem then yields

$L(f) = \lim_{\epsilon \rightarrow 0} L(f_\epsilon)$, where $L(f_\epsilon) = \sum_{k=-\infty}^{k=+\infty} \sigma_k L(\varphi_k(f)) = \sigma(f; \epsilon)$, as we wished to show.

If we apply these considerations to a particular instance of our general theory we obtain immediately an important theorem:⁴

- (18) *if E is any positive linear functional on the real vector-lattice \mathfrak{E} of*

all continuous real functions with compact nucleus on a locally compact space X , then E can be expressed as an integral in the sense of Lebesgue with respect to a measure on X .

As we have already pointed out, \mathfrak{E} and E satisfy our basic postulates and therefore lead to the introduction of an associated general integral and an associated outer measure. Since I (3) is valid here, we see that $1 \in \mathfrak{M}$ and hence that (15), (16) and (17) are valid also.

It is an open question to determine what modifications (if any) in our definitions and procedures will permit a more thorough analysis of the case where 1 is not measurable.

¹ Stone, M. H., "Notes on Integration, I," these PROCEEDINGS, **34**, 336-342 (1948); cited as I.

² Mazur, S., "Une remarque sur l'homéomorphie des champs fonctionnels," *Studia Math.*, **1**, 83-85 (1929).

³ Mr. H. Rubin, while a member of one of my classes, worked out much useful information on the subject of this paragraph and the next.

⁴ This result is closely related to theorems given by Riesz, F., "Sur certains systèmes singuliers d'équations intégrales," *Ann. Sci. de l'Ec. Norm. Sup.* (3), **28**, 33-62 (1911); Markoff, A., "On Mean Values and Exterior Densities," *Mat. Sbornik*, **4**, 165-191 (1938), especially Theorems 17 and 20; Kakutani, S., "Concrete Representation of Abstract M-Space," *Ann. Math.*, **42**, 994-1024 (1941), especially Theorem 9. The measure found in (18) is always regular in the sense of Carathéodory; but it is defined with certainty only for compact G_δ -sets and not necessarily for all compact sets (except, of course, when X is separable). This measure is therefore not necessarily identical with the one introduced by Markoff. The distinction is that between "Baire measures" and "Borel measures" (in the terminology of P. R. Halmos) and is known to be genuine on the basis of an unpublished example of J. Dieudonné. We shall have more to say about this situation in our fourth note.

SOME PRELIMINARY RESULTS ON THE SPECTRA OF AsH_3 , AsD_3 AND PH_3 *

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Measurements have been made on several of the bands in the spectra of AsH_3 , AsD_3 and PH_3 . Work is progressing on the analysis of these bands, but since some considerable time will be required to bring this work to completion we wish here to present a report on the work accomplished thus far on the spectra of these molecules.

Figure 1 represents the 4.5μ absorption region in the spectrum of AsH_3 gas. This is the region studied earlier by Lee and Wu¹ under a resolving

power somewhat smaller than ours. We believe this region to consist of the two fundamental bands ω_1 and ω_2 (using the notation of Dennison) where ω_1 is a vibration where the electric moment oscillates along the axis of symmetry and ω_2 is a twofold degenerate oscillation normal to the axis of symmetry. The sharp set of lines is taken to be those associated

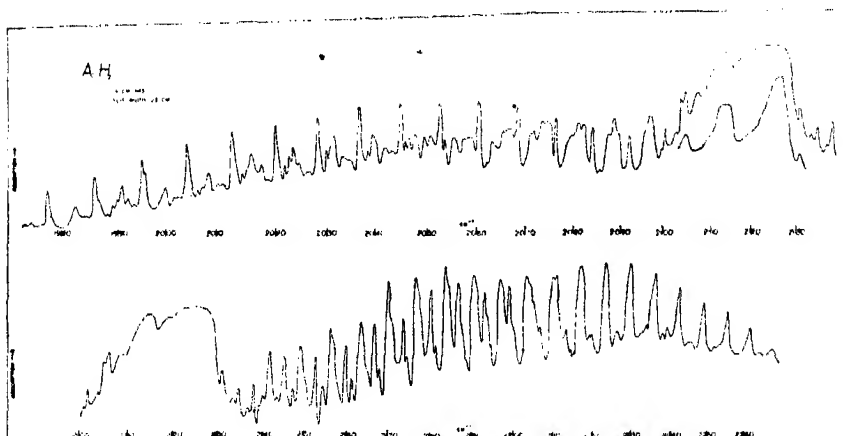


FIGURE 1

ω_1 and ω_2 in the infra-red spectrum of AsH_3 .

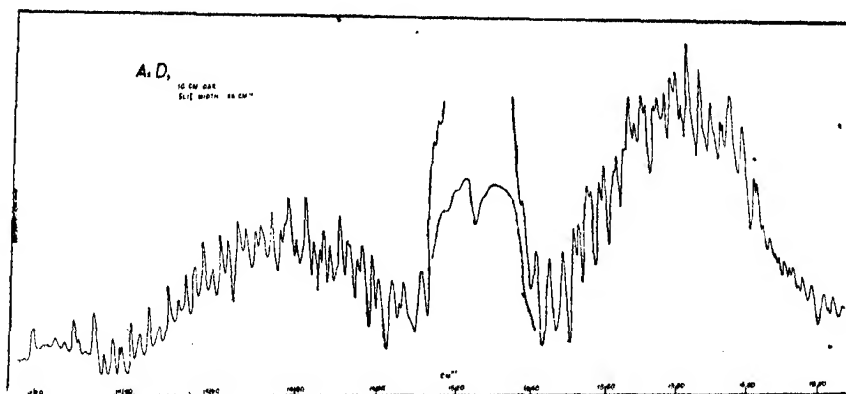


FIGURE 2

ω_1 and ω_2 in the infra-red spectrum of AsD_3 .

with ω_1 while the diffuse set (particularly so on the low-frequency side) are taken to be associated with ω_2 . An analysis of ω_1 has led to the values $B'' = 3.723 \text{ cm.}^{-1}$ and $B' = 3.682 \text{ cm.}^{-1}$, where the B 's are the reciprocals of inertia, $h/8\pi^2 c I_{\text{eff}}$, effective in the normal state and the vibration state ω_1 . The band center ν_1 is found to have the value 2116.1 cm.^{-1} .

Figure 2 represents the corresponding region in the spectrum of heavy arsine. An analysis of ω_1 in this spectrum leads to the values $B'' = 1.896 \text{ cm.}^{-1}$ and $B' = 1.883 \text{ cm.}^{-1}$ for the reciprocals of inertia of this molecule in its normal and excited states. The band center ν_1 for AsD_3 is found to be 1523.1 cm.^{-1} .

If the values Be (and therefore I_{xx}'') are known for both the molecules AsH_3 and AsD_3 it is possible to determine the two quantities $r_0(\text{As-H})$ and the angle between r_0 and the axis of symmetry, β . From these we may then determine the values I_{xx}'' and the reciprocals of inertia $Ce = h/8\pi^2 c I_{xx}''$. For Be we shall here use the values B'' for the molecules in the normal state. We obtain for r_0 the value $r_0 = 1.5126 \text{ \AA}$ and for the value $\beta = 54^\circ 39'$. For C'' the two values $C'' = 3.663 \text{ cm.}^{-1}$ and $C'' = 1.831 \text{ cm.}^{-1}$ are obtained, respectively, for the molecules AsH_3 and AsD_3 .

With these values for B'' and C'' an attempt has been made to analyze the band ω_2 for AsH_3 . The details of this will be deferred for a later communication, but satisfactory agreement is obtained with experiment when the values $\nu_2 = 2123 \text{ cm.}^{-1}$, $B' = 3.69 \text{ cm.}^{-1}$, $(1 - \zeta_2) C - B = 0.16 \text{ cm.}^{-1}$ and $(B' - B'' - C' + B'') = 0.02 \text{ cm.}^{-1}$ are adopted. These calculations lead to an approximate value of $\zeta_2 = -0.0602$ for AsH_3 .

Figure 3 shows the 10μ region of absorption for AsH_3 . This region we believe is also made up of two bands, namely, ω_3 and ω_4 using as before the notation of Dennison. ω_3 is a vibration which induces an electric moment parallel to the axis of symmetry. We identify the band center 905 cm.^{-1} with ν_3 because of the two Q branches and the band center 1002.6 cm.^{-1} as ν_4 . No attempt has been made to analyze either the bands ν_3 or ν_4 , but it is found that in the R branch of ν_4 and the P branch of ν_3 the spacings between the R and P lines are, respectively, about 10 cm.^{-1} as compared to a spacing of about 7.0 cm.^{-1} in the bands ν_1 and ν_2 . An explanation of this anomaly of rotational spacing is found in a strong Coriolis coupling between the frequencies ω_3 and ω_4 . The theory of such an interaction has been developed by one of us² and applied in a general way to the case of AsH_3 in an earlier note. An attempt to analyze this region in detail will be made at a later time.

The region corresponding to figure 3 for AsD_3 is not available. Measurements were made on this region, but it appears that the sample used for this set of measurements was contaminated with some of the hybrid molecules AsHD_2 and AsH_2D . This region we hope to repeat at a later date. For ν_3 and ν_4 for the AsD_3 molecule we take the values given by Lee and Wu.¹ Their values are $\nu_3 = 660 \text{ cm.}^{-1}$ and $\nu_4 = 714 \text{ cm.}^{-1}$.

If the normal frequencies ω_2 and ω_4 are known, and not just the band centers, it is possible to calculate ζ_2 for AsD_3 and compare the computed value with the experimental value. A method for closely simulating the normal frequencies for molecules like AsH_3 and AsD_3 has been given by

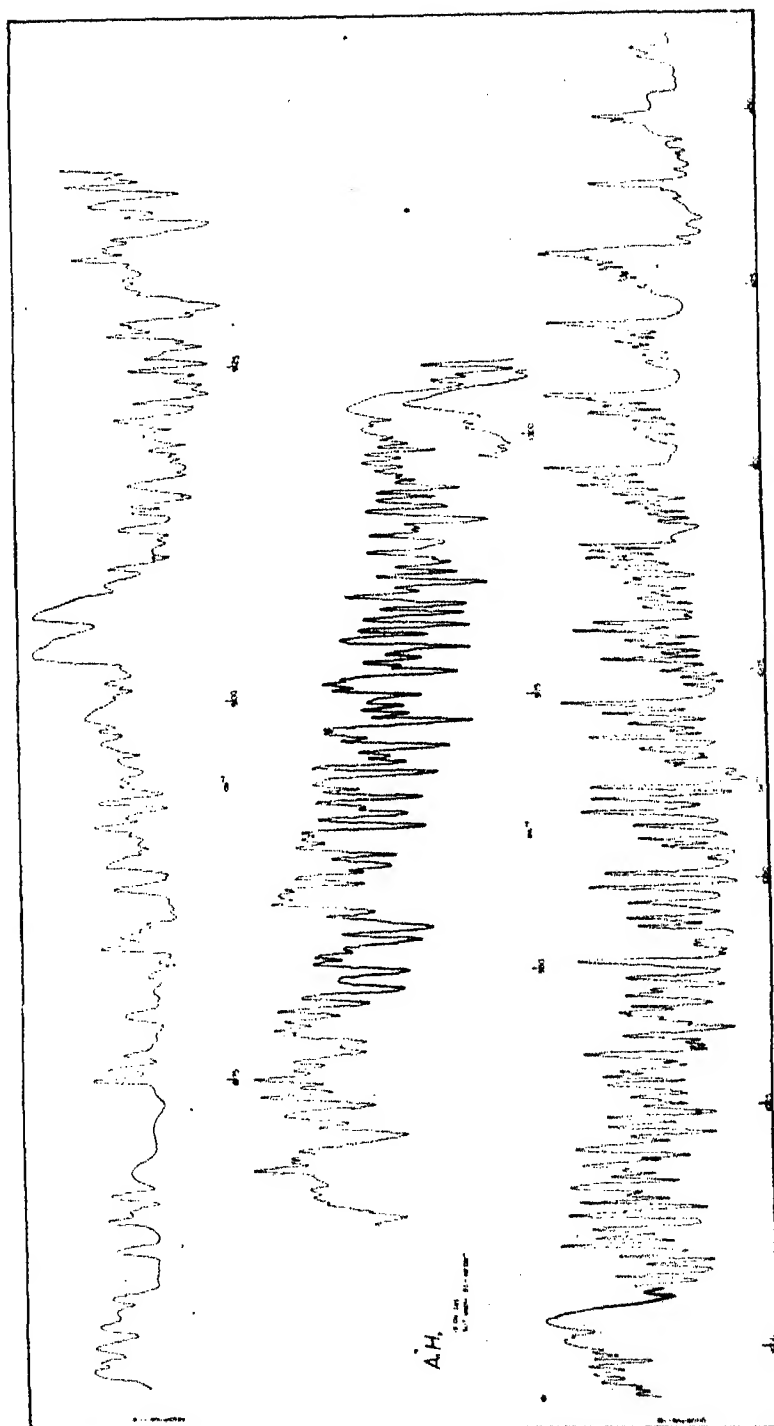


FIGURE 3

ω_2 and ω_1 in the infra-red spectrum of AsH_3 .

Dennison⁴ and this method is applied here. For ξ_2 for the AsD_3 molecule $\xi_2 = -0.0383$ is obtained. For the value $(1 - \xi_2)C - B$ a value 0.0053 cm.^{-1} is predicted. This value seems consistent with the measurements on ω_2 for that molecule which shows that the lines are about equally sharp on both the high- and the low-frequency side of the band. For this to be true $(1 - \xi_2)C - B$ itself must be very small as well as the value $B' - C' - B'' + C''$.

It will be seen that the angle β obtained for the AsH_3 molecule in this work is considerably smaller than the value derived by Sutherland, Lee and Wu.⁵ For this reason it is worth while to make an independent verification of this value. We may assume that the force fields of the molecule are closely approximated here by valence forces. On the basis of valence force fields Lechner⁶ has derived certain equations which relate the normal frequencies to the valence force constants, the atomic masses and the angle β . We state one of these

$$4\lambda_2\lambda_4 = \frac{f}{m} \frac{6d}{m'1 + 3 \cos^2} \left[\rho + (2 - \rho) \cos^2 \right] \quad (1)$$

where $\rho = \frac{3m + M}{M}$, M and m being the masses of the As and H atoms

TABLE 1
BAND CENTERS AND NORMAL FREQUENCIES FOR AsH_3 AND AsD_3

MOLECULE	ν_1	ν_2	ν_3	ν_4	ω_1	ω_2
			904.4			
AsH_3	2116.1	2123.0	906.9	1003	2225.8	1012.1
AsD_3	1523.1	1529.3	...	714	1528.4	718.6

TABLE 2
ROTATIONAL CONSTANTS AND MOLECULAR DIMENSIONS OF AsH_3 AND AsD_3

MOLECULE	$I_{zz} \times 10^{40}$	B_0	$I_{xx} \times 10^{40}$	C_0	r_0	h_0	
AsH_3	7.517	3.723	7.641	3.663	1.513 A	0.93 A	$54^\circ 39'$ $54^\circ 15'$
AsD_3	14.76	1.896	15.282	1.831	1.513 A	0.93 A	$54^\circ 39'$ $54^\circ 15'$

and where $\lambda_i = 4\pi^2 c^2 \omega_i^2$, ω_i being one of the normal frequencies. The normal frequencies ω_2 and ω_4 for the two molecules AsH_3 and AsD_3 have been found to take the values $\omega_2 = 2225.8 \text{ cm.}^{-1}$, $\omega_4 = 1012.1 \text{ cm.}^{-1}$, $\omega_2' = 1582.4 \text{ cm.}^{-1}$ and $\omega_4' = 718.6 \text{ cm.}^{-1}$. Since f , d and β will be the same in the two molecules one may quickly arrive at the relation

$$\frac{\lambda_2\lambda_4}{4\lambda_2'\lambda_4'} = \frac{\rho + (2 - \rho) \cos^2 \beta}{\rho' + (2 - \rho') \cos^2 \beta} \quad (2)$$

Using the above values for ω_i one quickly arrives at the value $\beta = 54^\circ 15'$ which is in satisfactory agreement with the value arrived at earlier. This

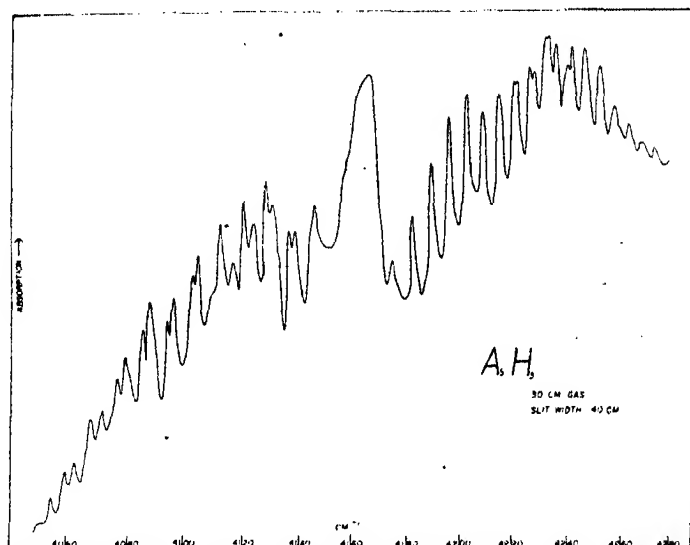


FIGURE 4

Absorption band in the spectrum of AsH_3 near 4160 cm^{-1} .

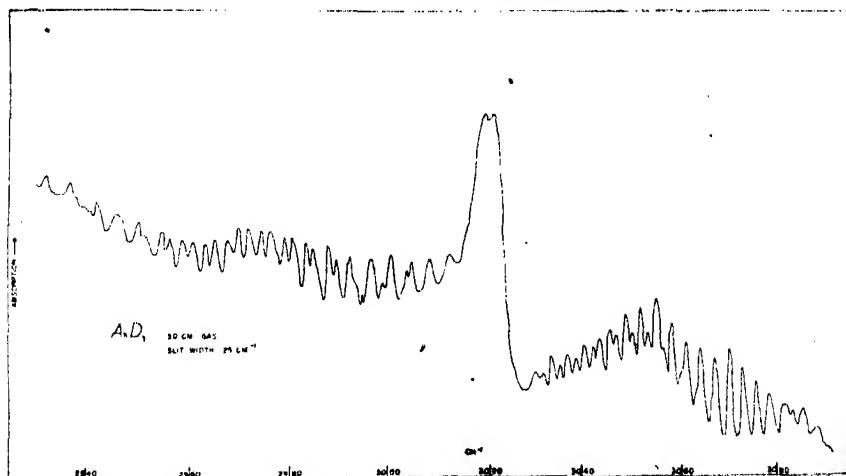


FIGURE 5

Absorption band in the spectrum of AsD_3 near 3020 cm^{-1} .

calculation serves to illustrate how important it is to use the normal frequencies rather than the band centers in determining the constants of a molecule from vibration spectra.

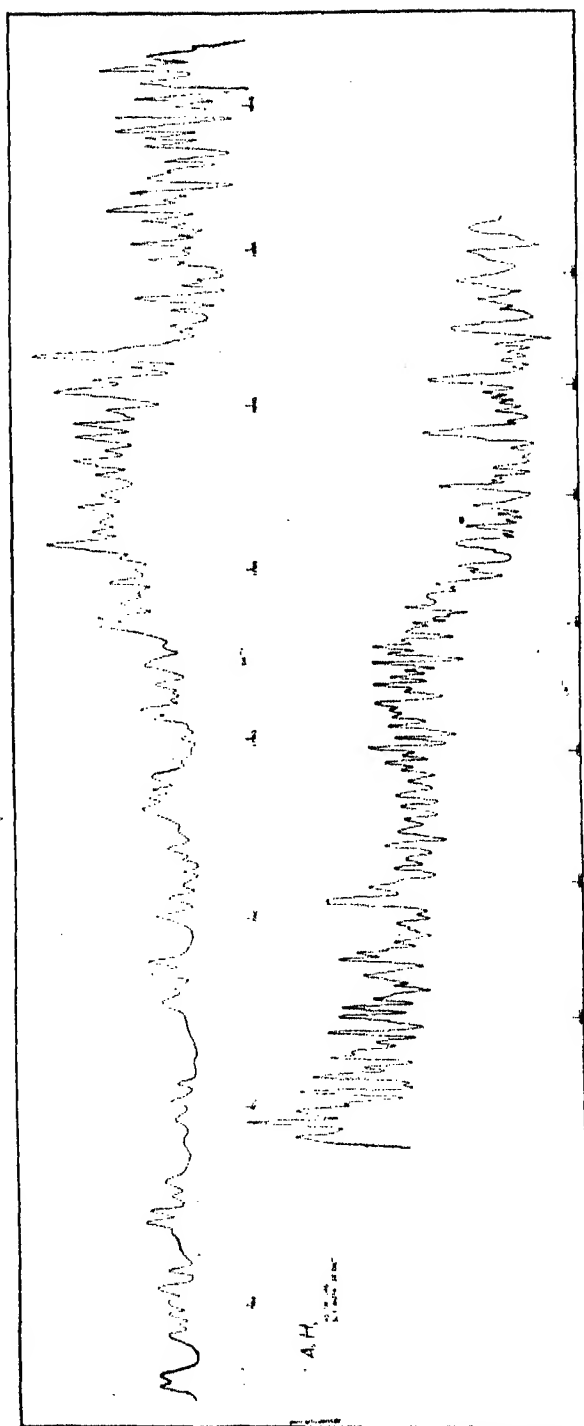


FIGURE 6

Absorption band in the spectrum of AsH_3 near 1800 cm^{-1} .

In tables 1 and 2 we summarize the results obtained for the AsH_3 and AsD_3 molecules.

Figures 4 and 5 are absorption regions in the spectrum of AsH_3 and AsD_3 . In each case there appears to be two independent and overlapping bands. If the combination relations are applied to the two sets of lines in each case the band centers seem to be located at 4174.2 cm.^{-1} , 4170 cm.^{-1} and 3022.2 cm.^{-1} , 3019.8 cm.^{-1} . While no definite identification is given here it is suggested that they may be $2\nu_2$ and $2\nu_1$, respectively, in each case. Figure 6 depicts an absorption region near 5.5μ . It resembles strongly the region near $10\text{--}11 \mu$ and it is suggested that it comprises the bands $2\nu_3$ and $2\nu_4$.

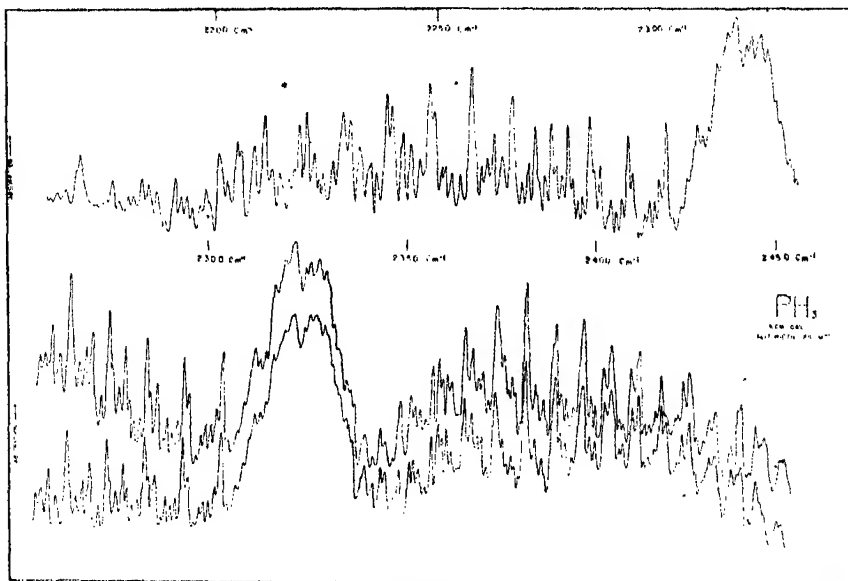


FIGURE 7

ω_1 and ω_2 in the infra-red spectrum of PH_3 .

Figures 7 and 8 represent diagrams of the principal absorption regions in the spectrum of PH_3 . The region near 4.3μ is the region studied by Fung and Barker.⁷ Their measurements covered only a part of the band because of the overlapping of the band by atmospheric CO_2 . We believe this region represents the two bands ν_1 and ν_2 . We believe the sharp lines to be associated with ν_1 and an analysis of these lines leads to a band center 2322.9 cm.^{-1} . The B values are found to be equal to $B' = 4.358 \text{ cm.}^{-1}$ and $B' = 4.301 \text{ cm.}^{-1}$. An analysis of the band ν_2 has not been carried out, but an estimate of the band center has been made. We take it to be 2328 cm.^{-1} .

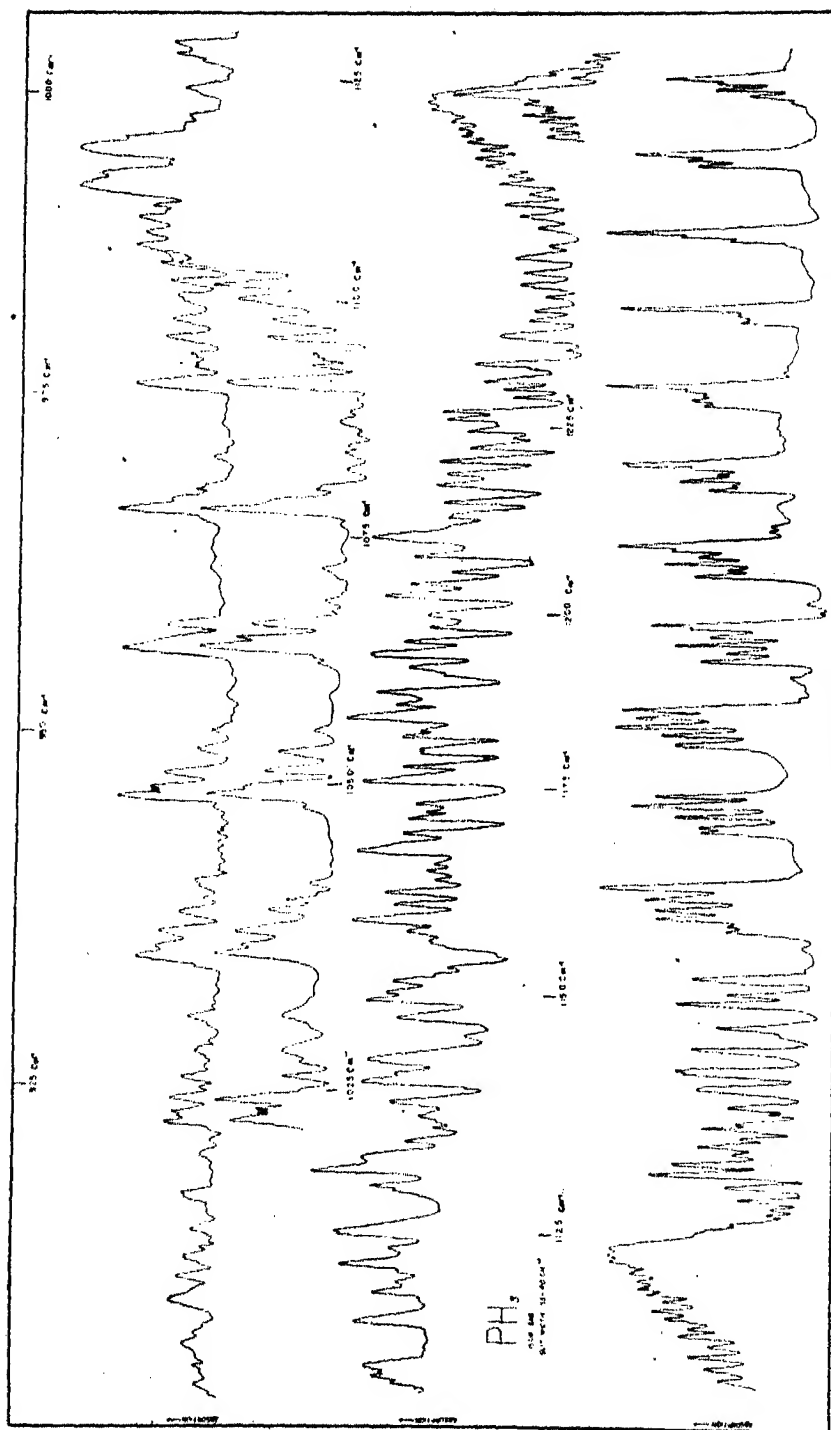


FIGURE 8

ω_1 and ω_4 in the infra-red spectrum of PH₃.

The region near $10\ \mu$ was also studied by Fung and Barker.⁷ Our dispersion seems considerably better than that available to them and we identify it as due to the vibrations ν_3 and ν_4 . We have estimated the centers to be $\nu_3 = 990\text{ cm.}^{-1}$ and $\nu_4 = 1121\text{ cm.}^{-1}$. Here as in the corresponding region in AsH_3 the spacing between rotational lines is anomalously large as compared to the rotational separation at $4.3\ \mu$. The effect is explained as in the case of AsH_3 as being due to a Coriolis interaction between ν_3 and ν_4 . The band ν_3 is double, as shown by Fung and Barker,⁷ the two Q branches being at 989.9 cm.^{-1} and 992.5 cm.^{-1} . It is a rather surprising fact that the band ν_3 in AsH_3 has a separation between the two components as large as that observed for PH_3 .

No records have been obtained at the present time on the spectra of PD_3 . It is hoped to obtain such records in the near future.

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* These results were discussed at the Symposium on Molecular Structure and Spectroscopy at Mendenhall Laboratory of Physics, Ohio State University, Columbus, Ohio, June 14-19, 1948.

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*EVIDENCE FOR AN INTERRELATION IN THE METABOLISM
OF LYSINE, ARGININE AND PYRIMIDINES IN NEUROSPORA**

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The behavior of a strain of *Neurospora crassa* carrying the pyrimidineless gene *3a* (37301) and *s*, a second mutant gene which suppresses the effect of *3a*, has been described briefly in an earlier publication.¹ A strain carrying *3a* alone has an absolute requirement for pyrimidine, while *3a-s* reaches about one-half the maximum growth of the wild type on the un-supplemented basal medium.² However, if arginine is present in this medium the pyrimidine requirement is again manifested. The effect of arginine may be suppressed in turn by the addition of lysine. This response to arginine and lysine suggested the possibility that one or both of these amino acids are involved in the biosynthesis of pyrimidines. More recent investigations have provided evidence that a relationship does exist, but its nature is not yet understood.

Of the two lines of evidence which indicate this interrelation perhaps the most convincing is the fact that two of four non-allelic lysineless mutants³ accumulate pyrimidine compounds in their culture media. One of the compounds has been isolated and identified as uracil. A second substance, more active biologically, has been shown to be present but has not been isolated. There is some evidence which suggests that this compound is uridine.

A second line of evidence is based on growth responses of multiple mutant strains derived from crosses of lysineless mutants to pyrimidineless *3a-s*. The unexpected behavior of several of these strains has not been satisfactorily interpreted, but, in the opinion of the authors, such an interpretation must involve an interrelation in the metabolism of pyrimidines and lysine, and, possibly of arginine as well.

Accumulation of Pyrimidines by Lysineless Strains.—Four lysineless

mutants, shown by Doermann³ to be non-allelic, have been tested. The isolation numbers are 33933, 37101, 4545 and 15069; for simplicity they will be called *ly* 1, 2, 3 and 4. After the finding by Borsook, *et al.*,⁴ that lysine is converted to α amino adipic acid by mammalian tissue slices and homogenates it was shown that this amino acid can be used as a substitute for lysine by strain *ly* 1 but not by *ly* 2, 3 and 4.⁵ From this it is inferred that the reaction blocked in strain *ly* 1 precedes those blocked in the other strains.

Accumulated pyrimidine compounds have been demonstrated in culture fluid from *ly* 3 and 4 but not in that from *ly* 1 and 2. The amount found, in terms of cytidine sulfate activity for the pyrimidineless strain 3a, varied from 0.1 to 0.2 mg. in 10 ml. of culture fluid. The substances promote growth of three non-allelic pyrimidine-requiring strains, 37301 or 3a, 263 and 38502.⁶ The accumulation was observed in four-day cultures containing 1 mg. of L(+) lysine monohydrochloride in 20 ml. of medium. This quantity of lysine is sufficient to allow half-maximum growth of the lysineless strains. When higher lysine concentrations were used little or no pyrimidine activity was detected.

Of the four lysineless strains, 1 and 3 have been more thoroughly tested. In repeated experiments *ly* 3 has not failed to show the accumulation under the conditions given above while *ly* 1 has always failed to do so. Moreover, no accumulation by the double mutant *ly* 1-*ly* 3 has been observed. In three experiments with *ly* 2 and *ly* 4 the former has behaved like *ly* 1 and the latter like *ly* 3. Culture fluid from wild type (Abbott 4) and from an adenine-requiring strain 27663-44206 supplied a limiting quantity of adenine, showed no biological activity for the pyrimidineless strains.

Uracil was isolated from a culture of *ly* 3 grown four days at 25°C. in a five-gallon bottle containing 15 l. of basal medium supplemented with 750 mg. of L(+) lysine monohydrochloride. The material was first concentrated by adsorption on Norite (2 mg. per ml. of culture fluid) and elution with boiling 10% aniline solution. The active compounds were then precipitated by the addition of excess silver nitrate at about pH 8. Silver was removed as the sulfide, the volume reduced by evaporation and crystalline compound was obtained. After two recrystallizations from hot water the compound melted with decomposition at 335°C. (corr.). Literature values for uracil are 338°C., 335°C. The ultra-violet absorption spectrum was identical to that of uracil and the biological activity for *pyr* 3a was also the same.

It seems clear that all of the biological activity found is not due to uracil. The activity of a number of partially purified preparations exceeded by a considerable margin that of the quantity of uracil which will remain dissolved in the same volume of water. Moreover, solutions of such highly active preparations showed the maximum absorption of ultra-violet light at the same wave-length (259 m μ) in 0.1 N NaOH as in 0.1 N HCL. This

is characteristic of the spectrum of uridine, while solutions of uracil, on the other hand, show a change from 259 $m\mu$ in acid to 283 $m\mu$ in alkali.

It was found that a small quantity of cytidine, which has the same biological activity as uridine (about 30 times that of uracil⁶) will greatly enhance the activity of uracil. A mixture of 0.1 mg. of cytidine sulfate and 1.6 mg. of uracil is equivalent in activity to 8 mg. of uracil alone. It seems safe to conclude, therefore, that a pyrimidine derivative with the activity of uridine or cytidine is also accumulated. The ultra-violet absorption spectra mentioned above indicate that it is uridine.

TABLE 1
REQUIREMENTS FOR HALF-MAXIMUM GROWTH OF SINGLE AND MULTIPLE MUTANT STRAINS

STRAIN	REQUIREMENT—MICROMOLS/20 ML. MEDIUM
<i>s</i>	0
<i>pyr 3a</i>	cytidine sulfate 1.6; uracil 54.0 cytidine sulfate 0.31 + uracil 2.7
<i>pyr 3a-s</i>	0
<i>ly 1</i>	L-lysine 5.5 DL α amino adipic acid 6.2
<i>ly 1-s</i>	L-lysine 5.5 DL α amino adipic acid 25.0 DL α amino adipic acid 6.2 + L-arginine 0.47 DL α amino adipic acid 6.2 + DL citrulline 5.7 DL α amino adipic acid 6.2 + DL ornithine 13.0
<i>ly 1-pyr 3a</i>	L-lysine 5.5 + cytidine sulfate 1.6 DL α amino adipic acid 6.2 + cystidine sulfate 1.6 + L-lysine 0.55
<i>ly 1-pyr 3a-s</i>	L-lysine 5.5
<i>ly 3</i>	L-lysine 5.5
<i>ly 3-s</i>	L-lysine 5.5
<i>ly 3-pyr 3a</i>	L-lysine 5.5 + cytidine sulfate 1.6
<i>ly 3-pyr 3a-s</i>	L-lysine 5.5 + cytidine sulfate 1.6 + L-arginine 2.4

Double and Triple Mutants Involving Ly 1, Pyr 3a and s.—The requirement for lysine due to the mutant gene *ly 1* is not affected by the presence, in the same strain, of *s* or *pyr 3a*, nor is the pyrimidine requirement of *pyr 3a* altered by the presence of *ly 1*. Growth of the triple mutant *ly 1-pyr 3a-s*, when supplied adequate lysine, parallels that of *pyr 3a-s* on the unsupplemented basal medium. However, with respect to their ability to use α amino adipic acid as a substitute for lysine each of the three strains; *ly 1-s*, *ly 1-pyr 3a* and *ly 1-pyr 3a-s* differs from strain *ly 1*.

The quantity of α amino adipic acid required to initiate growth of strain *ly 1-s* is about four times that which is necessary for half-maximum growth of *ly 1*, but if arginine, citrulline or ornithine is added then the response of this strain is very nearly like that of the single mutant. Citrulline is less effective than arginine and ornithine is still less effective; also the effect of the latter is rather erratic. The quantities required are given in table 1.

This response to these amino acids is of particular interest because of their effect upon the lysineless strains and upon *pyr 3a-s*. Their presence in the culture medium inhibits the utilization of lysine by all of the lysineless strains⁷ and prevents growth of strain *pyr 3a-s* in the absence of pyrimidine.¹ The relative effectiveness of arginine, citrulline and ornithine is the same in the three cases.

Strain *ly 1-pyr 3a* does not grow when supplied α amino adipic acid with cytidine and cannot be made to do so by the addition of arginine, citrulline or ornithine. However, if a small quantity of lysine is added, about one-tenth the requirement for half-maximum growth, then, in the presence of adequate cytidine, the response of this strain to α amino adipic acid is like that of *ly 1*. Utilization of this compound by *ly 1-s* is apparently not affected by lysine. When supplied lysine in addition to quantities of α amino adipic acid smaller than that required to initiate growth, this strain responds as it does to the same quantity of lysine alone.

Utilization of α amino adipic acid by *ly 1-pyr 3a-s* has not been demonstrated although the following additions have been tried: lysine; arginine plus cytidine; and lysine plus arginine plus cytidine.

No inhibitory effects have been observed of cytidine upon the utilization of α amino adipic acid by strain *ly 1*, nor of this amino acid upon the utilization of cytidine by *pyr 3a*. Growth of strain *pyr 3a-s* is not affected by α amino adipic acid.

Double and Triple Mutants Involving Ly 3, Pyr 3a and s.—Requirements for lysine and cytidine of the double mutants *ly 3-s* and *ly 3-pyr 3a*, like the corresponding combinations with *ly 1*, have not been found to differ from those of the single mutants. Strain *ly 3-pyr 3a-s*, on the other hand, departs markedly from the behavior which would be predicted for it on the basis of characteristics of the single and double mutants involving these three genes. It does not grow when supplied lysine alone, but requires pyrimidine and arginine as well. Moreover, in order to allow maximum growth, lysine and arginine must be supplied in a molar ratio of 2.3 to 1. The requirement for pyrimidine is not surprising since the suppressor does not function if arginine is present unless the lysine concentration is about six times that of arginine. When lysine and arginine are supplied in suitable concentrations then the response of this strain to cytidine is the same as that of *pyr 3a*. Table 1 lists the requirements for half-maximum growth of the various single and multiple mutant strains.

There are two points of similarity between *ly 3-pyr 3a-s* and the arginineless mutant 36703. First, neither citrulline nor ornithine will satisfy the arginine requirement.⁸ Second, both strains are inhibited by lysine if the molar ratio of lysine concentration to arginine concentration is 3.5 to 1 or greater. For this reason strains of the constitutions *arg-s* and *arg-pyr 3a* were considered of possible interest and were prepared. However, no

departures from predicted behavior have been observed. *Arg-s* responds to arginine and lysine as does *arg* alone, while requirements of *arg-pyr 3a* for arginine and cytidine are like those of *arg* and *pyr 3a*. Combinations involving *arg* and *ly 1* or *ly 3* may prove of interest but they have not yet been obtained.

Verification of Genetic Constitution of Multiple Mutant Strains.—The various double and triple mutants were obtained from crosses of *pyr 3a-s* to *ly 1*, *ly 3* and *arg*. *Ly 1-s*, *ly 3-s* and *arg-s* were selected from asci containing four spores carrying *pyr 3a* alone. The other four spores would, of course, carry *s* along with the third mutant gene involved in the cross. The double mutants of *pyr 3a* with *ly 1*, *ly 3* or *arg* were obtained from asci which gave rise to four wild-type appearing strains and four having the appropriate double requirement, lysine plus pyrimidine or arginine plus pyrimidine. *Ly 1-pyr 3a-s* came from an ascus from which were obtained four wild-type appearing strains and four requiring only lysine. The ascus from which *ly 3-pyr 3a-s* was derived gave rise to four wild-type strains and four which failed to grow when supplied lysine plus cytidine. The identity of these two strains was further checked by recovering from crosses to wild-type strains carrying *pyr 3a-s* and others requiring lysine alone or lysine plus pyrimidine. *Arg-pyr 3a* had, of course, to be crossed to wild type in order to distinguish it from *arg-pyr 3a-s*.

In the case of each multiple mutant described, isolates from several different asci were tested and found to have the same characteristics.

Discussion.—The fact that obstruction of the biosynthesis of lysine at either of two points results in accumulation of pyrimidines, while interruption of the series at two other points does not produce this result, establishes an interdependence in the metabolism of pyrimidine and lysine in *Neurospora*. Such a relationship is also strongly indicated by the fact that introduction of a genetic block in the series of reactions by which the pyrimidine ring is synthesized interferes with the utilization of a precursor of lysine, α amino adipic acid, by strain *ly 1-pyr 3a*. The behavior of *ly 1-s* and *ly 1-pyr 3a-s*, as well as that of *ly 1-pyr 3a*, suggests that utilization of this compound is dependent upon the *pyr 3a* reaction.

No satisfactory scheme has been devised which will explain how lysine metabolism is related to pyrimidine biosynthesis and in what way arginine is involved.

Summary.—Of four non-allelic lysineless strains two have been shown to accumulate pyrimidines, while two do not. One of the accumulated compounds has been isolated and identified as uracil. Another has been tentatively identified as uridine.

The double and triple mutant combinations of the pyrimidineless gene, *3a* and its suppressor, *s*, with two lysineless strains have been prepared and tested. Several of these show growth responses, not characteristic of the

single mutants involved, which indicate a relationship in the metabolism of pyrimidines, lysine and arginine. Combination of the genes, lysineless 3, pyrimidineless 3a and *s* introduces a requirement for arginine. Lysineless strain 1 is able to use α amino adipic acid as effectively as it uses lysine. Combination of this gene with the suppressor gene *s* produces a strain which has the same requirement for lysine as lysineless 1, but a much higher requirement for α amino adipic acid, unless arginine, citrulline or ornithine is also supplied. By combination of this lysineless gene with pyrimidineless 3a a strain is produced which cannot use α amino adipic acid unless a small amount of lysine is added.

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THE CYTOGENETIC EFFECT OF SONIC ENERGY APPLIED SIMULTANEOUSLY WITH X-RAYS*

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Communicated by Karl Sax, July 23, 1948

It is generally agreed that the number of chromosome breaks initially produced by a given dose of x-rays is independent of conditions at the time of radiation. But the subsequent yield of observable chromosomal aberrations from a given number of initial breaks depends upon the ratio of those breaks which rejoin (and are not detectable) to those which do not rejoin or which rejoin in detectable new associations. The majority of the breaks produced by x-radiation, under normal conditions, seem to rejoin in their original position and do not form observable aberrations.¹

Any factors which affect this process of rejoining will influence the yield of chromosomal aberrations obtained from a given x-ray dose. Several treatments used in conjunction with x-rays have been shown to increase (or decrease) the yield as compared with that obtained from the same dose

of x-rays alone. The results of some of these treatments are summarized in table 1:

TABLE 1
EFFECT ON X-RAY ABERRATION FREQUENCY

TREATMENT	EFFECT ALONE	BEFORE	DURING	AFTER	REFERENCE
Centrifugation	0	x	+ (2/1)*	0	(5)
Temperature (3°/36°)	0	0	+ (4/1)	0	(4)
Colchicine	0	— (1/3)	x	x	(2)
Ultra-violet	+	— (1/2.5)	x	— (1/2)	(8)
Infra-red	0	+ (2/1)	x	+ (2/1)	(9)

* Ratio, x-ray + treatment/x-ray alone.

x = not studied.

In addition to the treatments above, the effect of the stage of division at the time of radiation, genetic differences and differences between different tissues of the same individual have been studied.⁶

Most of these artificial or natural influences (except ultra-violet and infra-red) seem to produce their effect on the yield of chromosomal aberrations by altering somehow the amount or freedom of chromosome movement. An increase in the amount of movement after chromosomes have been broken by x-rays would enable the broken ends from one break to be separated from one another (reducing restitution) and would bring broken ends from different breaks together (increasing new reunions); either would increase the yield of aberrations. These considerations, and the work which had been done with centrifugation, suggested that sonic or supersonic energy would be a means of applying mechanical agitation to cellular structures during radiation, thereby increasing the yield of chromosomal aberrations.

Experimental Methods.—Inflorescences of a clone of *Tradescantia paludosa* Anderson and Woodson were subjected to simultaneous x-ray and sonic treatment. Aceto-carmin smears of the anthers were made four days after treatment, and the frequency of chromosomal aberrations determined at the microspore mitosis. X-rays were delivered at 250 kv. peak, 15 ma., filtered through $\frac{1}{2}$ mm. Cu and 1 mm. Al. The average intensity, measured with a Victoreen dosimeter in air at the same position as the buds were when exposed to radiation, was 78 r/minute. A dose of 250 r was given in all experiments.

Sonic treatment was begun with the x-radiation and continued for five minutes after radiation ceased. The sonic treatment was administered by a Raytheon Manufacturing Company Model R-22-3 magnetostriction transducer connected to an electronic oscillator operating at about 9100 cycles/second.⁷ The material was treated in a stainless steel cup, the bottom of which was a diaphragm connected directly to a nickel magneto-

striction rod which alternately contracted and expanded in an alternating electromagnetic field. The sound energy generated by the cup diaphragm was transmitted to the *Tradescantia* buds by water 3.4 cm. deep (30 cc.) in which the inflorescences were immersed, upside down, the center of the buds being 3 mm. beneath the surface of the water through which the sonic vibrations were transmitted. Temperature was kept constant by a continuous flow of tap water (18°C.) through the water jacket of the double-walled sonic cup. Inflorescences were held in place during treatment by inserting the stem base of the inflorescence through a hole in a thin lucite cap fitted over the top of the cup. The machine was always operated at the same voltage level across the transducer, 30 volts, and at the same frequency, about 9100 cycles/second. The sonic cup, with the flower buds held in position in the water, was centered under the x-ray tube; in both the control series (x-rays only) and the treated series (x-ray plus sonic energy), buds were held in the same position in the water inside the sonic cup during treatment. The relative x-ray dose received by the buds in the sonic and control series was the same; however, the absolute dose, due to scattering by the lucite cap, water and steel cup, may not have been the same as the 250 r measured by a dosimeter in the same position in air.

Results.—A series of six experiments was made. Since the results of all are essentially the same, the data have been combined and are presented in table 2.

TABLE 2
YIELD OF FOUR-DAY CHROMOSOMAL ABRERRATIONS PER 100 CELLS. 250 R X-RAYS
(AV. 78 R/MIN.); AV. 7½ MINUTES SONIC TREATMENT

TREATMENT	NO. OF BUDS	NO. OF CELLS	EXCHANGES*		DELETIONS†	
			NO.	PER 100 CELLS	NO.	PER 100 CELLS
X-rays + sonic	21	1481	547	36.9 ± 1.4	751	50.7 ± 1.9
Control (x-ray alone)	8	650	189	29.1 ± 2.0	261	40.2 ± 2.5
Diff. (sonic) — (x-rays) ± S.E. diff.	7.8 ± 2.5	...	10.5 ± 3.2
Ratio: sonic/x-ray	1.27/1	...	1.26/1

* Dicentrics + tracentrics + centric rings.

† Terminal + small isodiametric fragments (acentric rings excluded).

Previous experiments had shown that the sonic treatment alone, as given here, did not cause detectable chromosomal aberrations.† The yield of x-ray induced aberrations has been increased by the application of sonic energy; the differences between the sonic treated and x-ray controls are statistically significant for both the one- and two-hit aberrations (difference > 3 times S.E. diff.).

Discussion.—The increase in aberration yield is somewhat less than that obtained by other treatments, where the ratio of treated/control has been

about 2/1 for centrifugation, and infra-red, about 4/1 for cold treatment. It is believed that the smaller effect found with sonic energy, as administered in these experiments, is due to the fact that it is difficult to deliver a sonic dose intense enough to produce the effect desired without causing a disruption of the cells or entire tissue, making further observation impossible. An intensity of 30 volts across the transducer was empirically determined as the maximum intensity which could be used, and still have the buds and anthers survive intact. In these experiments, cells have been observed as much as five days after treatment in which the entire nucleus had been disrupted into numerous small droplets scattered throughout the cell, in other cases the nucleus had been condensed into a solid, amorphous pycnotic globule. It is interesting that some cells, five days after treatment, though still intact and at the same stage of cellular development as normal cells, had no vestige of the nucleus or nuclear fragments remaining, though the cytoplasm was uniformly darker stained than nearby normal cells. Apparently the nucleus was almost completely dispersed throughout the cells, yet five days of development and growth of the microspore ensued in such cells with a dispersion rather than an organization of genic material. There can be no doubt, however, that the transmission of sonic energy does cause movement of cellular constituents without disrupting the cell; such effects have been described in early biological studies made with sonics and supersonics.¹ Further, an x-ray induced chromosome fragment, because of its smaller mass and surface area, should have a smaller viscous drag and therefore be displaced by sonic vibration more than the heavier and larger chromosome from which it came. This may be observed qualitatively for isodiametric deletions ("minutes" or "dots") which, following x-ray treatment alone, are generally associated quite closely with the chromosomes; after combined x-ray and sonic treatment, the deletions are often completely dissociated from the chromosomes.

It is thought that by refinements in the method of treatment, and perhaps by using ultrasonic energy of much shorter wave-length than used here (9100 cycles/second \approx about 16 cm. wave-length in water) greatly enhanced yields of aberrations may be obtained; if the problem of inducing considerable vibrational movement of cellular constituents without causing disruption of the cell wall can be solved, this should be possible. Ultrasonics with wave-lengths of the order of magnitude of cell dimensions may be the way of doing this.

Summary.—The cytogenetic effects of simultaneous treatment of *Tradescantia* inflorescences with x-rays (250 r) and sonic energy (9100 cycles/second) are described. Simultaneous treatment with sonic energy and x-rays increases the yield of x-ray induced chromosomal aberrations about 1.3 times the yield obtained with the same amount of x-rays alone.

The sonic treatment alone did not cause aberrations. The increased yield is probably due to an increased movement of chromosomes and chromosome fragments caused by the sonic treatment, resulting in a decrease in the amount of restitution and an increase in the amount of detectable new reunions between the broken ends of chromosomes.

† A recent experiment¹⁰ reports the production of chromosomal aberrations, mutations and other effects in Allium root tips, Helianthus stem apices, and Drosophila by treatment with ultrasonics alone. However, the treatment was quite different in frequency (400,000 cycles/second) and in other factors from the sonic exposures reported here.

* This work was performed under Contract No. W-7405-Eng-26.

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RAYLEIGH-RITZ AND A. WEINSTEIN METHODS FOR APPROXIMATION OF EIGENVALUES.* I. OPERATORS IN A HILBERT SPACE

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The well-known method of Rayleigh-Ritz¹ permits us to find upper bounds for the eigenvalues of a differential operator. In the late thirties, A. Weinstein introduced a new method, in some cases important for applications, giving lower bounds for the eigenvalues.²

We were able to extend Weinstein's method, making it more precise and simplifying it at the same time by the use of the Hilbert space theory.

This method is seen then as a counterpart of the Rayleigh-Ritz method, or more precisely, of a generalized Rayleigh-Ritz method for which similar developments to Weinstein's method can be established.

In the present paper we develop the theory for completely continuous, symmetric operators in a Hilbert space. In another paper we shall study the applications of this theory to differential self-adjoint operators.

1. Consider in a Hilbert space \mathcal{H} , with the scalar product (x, y) , an operator H , symmetric and completely continuous.

For any linear closed subspace $\mathcal{L} \subset \mathcal{H}$ we denote by P the corresponding projection.

The operator $L = PH$, considered in the subspace \mathcal{L} , will be called *the part of H in \mathcal{L}* . It is a symmetric and completely continuous operator (in \mathcal{L}). If H is positive, L is positive too.

The eigenvalues, eigenvectors, resolvent operator, etc., of L (in \mathcal{L}) will be called the eigenvalues, eigenvectors, resolvent operator, etc., of H in \mathcal{L} .

An eigenvector u and the corresponding eigenvalue λ of H in \mathcal{L} satisfy the equation

$$Hu - \lambda u = p \quad \text{with } p \perp \mathcal{L}. \quad (1)$$

The resolvent operator R_λ of H in \mathcal{L} , for any $f \in \mathcal{L}$ satisfy the equation

$$HIR_\lambda f - \lambda R_\lambda f = f + p \text{ for } p \perp \mathcal{L}. \quad (2)$$

2. The eigenvalues of H in \mathcal{L} can be defined by maximum-minimum problems for the positive eigenvalues and by minimum-maximum problems for the negative eigenvalues.³ This is done in the following way. We define

$$Q(u) = \frac{(Hu, u)}{(u, u)} \text{ for } u \neq 0 \quad (3)$$

$$Q(0) = 0$$

and then for any set of n vectors ($n = 0, 1, 2, \dots$), $\varphi_1, \varphi_2, \dots, \varphi_n$ of \mathcal{H} we consider the maximum (or minimum) of $Q(u)$ for all vectors $u \in \mathcal{L}$ satisfying the conditions $(u, \varphi_k) = 0, k = 1, 2, \dots, n$. This maximum (or minimum) is attained by some vector and will be denoted by $\lambda_n\{\varphi_k\}$ (or $\mu_n\{\varphi_k\}$). If we vary the n vectors φ_k , the $\lambda_n\{\varphi_k\}$ will attain its minimum λ_n which is the n th positive eigenvalue of H in \mathcal{L} . In the same way, the minimum $\mu_n\{\varphi_k\}$, for varying φ_k , will attain its maximum μ_n which is the n th negative eigenvalue of H in \mathcal{L} . In this way we get the positive and negative spectra of H in \mathcal{L} :

$$\begin{aligned} \lambda_0 \geq \lambda_1 \geq \dots \rightarrow 0 & \quad \text{positive spectrum,} \\ \mu_0 \leq \mu_1 \leq \dots \rightarrow 0 & \quad \text{negative spectrum.} \end{aligned} \quad (4)$$

In this definition we do not exclude the vector $u = 0$. This fact makes it necessary to accept the following convention. If there are only a finite number of positive (or negative) eigenvalues, then the positive (or negative) spectrum has to be completed by an infinite number of zeros so that the sequences (4) will always have an infinite number of terms.⁴ The eigenvectors corresponding to the eigenvalues (4) will be denoted by

$$\begin{aligned} u_0^+, u_1^+, \dots \\ u_0^-, u_1^-, \dots \end{aligned} \quad (5)$$

When, following our convention, we continue the positive (or negative) spectrum by zeros, the corresponding u_n^+ or u_n^- will be equal to zero. All the other eigenvectors will be supposed normalized ($\|u_n^+\| = \|u_n^-\| = 1$). The operator H may admit in \mathcal{L} the eigenvalue 0. A corresponding complete system of orthonormal eigenvectors will be denoted by

$$u_1^0, u_2^0, u_3^0, \dots \quad (5')$$

3. The following two theorems are well known.⁵

THEOREM A. If $\mathcal{L} \subset \mathcal{L}'$ then for the corresponding eigenvalues we have

$$\lambda_k \leq \lambda'_k, \mu_k \geq \mu'_k, k = 0, 1, 2, \dots$$

THEOREM B. If $\mathcal{L} \subset \mathcal{L}'$ and $\mathcal{L}' \ominus \mathcal{L}$ is n -dimensional, then

$$\lambda'_k \geq \lambda_k \geq \lambda'_{k+n}, \mu'_k \leq \mu_k \leq \mu'_{k+n}, k = 0, 1, \dots$$

The following theorem seems new even in the case of an n -dimensional space, $n < \infty$.

THEOREM I. If $\mathcal{L}' \subset \mathcal{L}$, $\mathcal{L}'' = \mathcal{L} \ominus \mathcal{L}'$, we have the inequalities:

$$\lambda_{i+j} + \mu_0 \leq \lambda'_i + \lambda''_j, \mu_{i+j} + \lambda_0 \geq \mu'_i + \mu''_j, \text{ for } i, j = 0, 1, 2, \dots$$

An interesting special case of this theorem is

COROLLARY I'. Under the assumptions of Theorem I, if H is positive definite we have $\lambda_{i+j} \leq \lambda'_i + \lambda''_j$.

A sequence of subspaces $\mathcal{L}^{(n)}$ is said to converge to a subspace \mathcal{L} if for every $u \in \mathcal{H}$, $P^{(n)}u \rightarrow Pu$. We then have the theorem

THEOREM II. If $\mathcal{L}^{(n)} \rightarrow \mathcal{L}$ then $\lambda_k^{(n)} \rightarrow \lambda_k$, $\mu_k^{(n)} \rightarrow \mu_k$.

If $\mathcal{L}^{(n)}$ is an increasing (or decreasing) sequence of subspaces, then it converges to the smallest closed subspace \mathcal{L} containing all of them (or the intersection \mathcal{L} of all of them). It follows from theorems A and II that

COROLLARY II'. If $\mathcal{L}^{(n)} \nearrow \mathcal{L}$ (or $\mathcal{L}^{(n)} \searrow \mathcal{L}$), $\lambda_k^{(n)} \nearrow \lambda_k$, $\mu_k^{(n)} \searrow \mu_k$ (or $\lambda_k^{(n)} \searrow \lambda_k$, $\mu_k^{(n)} \nearrow \mu_k$).

4. Consider two subspaces, $\mathcal{L}' \subset \mathcal{L}$ with $\mathcal{L} \ominus \mathcal{L}'$ n -dimensional. Consider further, a system of n vectors p_1, p_2, \dots, p_n generating the subspace $\mathcal{L} \ominus \mathcal{L}'$, the resolvent operator R_λ of H in \mathcal{L} and

$$u_m(\lambda) = R_\lambda p_m, \quad m = 1, 2, \dots, n, \quad \lambda \text{ any complex number.} \quad (6)$$

We will denote by $W(\zeta)$ (Weinstein's determinant) the n th order determinant

$$W(\zeta) = \det. \{(u_m(\zeta), p_k)\} \quad (7)$$

and by $\Gamma \equiv \Gamma\{p_k\}$, the Gramm's determinant

$$\Gamma\{p_k\} = \det. \{(p_m, p_k)\}. \quad (8)$$

We have then the following fundamental theorem.

THEOREM III. *The function $\Phi(\zeta) = \frac{W(\zeta)}{\Gamma\{p_k\}}$ is dependent only on the operator H , and the subspaces \mathcal{L} and \mathcal{L}' . $\Phi(\zeta)$ is meromorphic in the whole plane ζ with the exception of $\zeta = 0$ (where it may have an essential singularity), is regular at $\zeta = \infty$ and representable by the product*

$$\Phi(\zeta) = \left(\frac{-1}{\zeta}\right)^n \prod_{k=0}^{\infty} \frac{(\zeta - \lambda'_k)}{(\zeta - \lambda_k)} \prod_{k=0}^{\infty} \frac{(\zeta - \mu'_k)}{(\zeta - \mu_k)}, \quad (9)$$

where $\lambda_k, \mu_k, \lambda'_k, \mu'_k$ are the eigenvalues of H in \mathcal{L} and \mathcal{L}' , respectively.

Consider now the projection P' on \mathcal{L}' and the resolvent operator R'_λ of H in \mathcal{L}' . We write

$$w_k(\lambda) = -R'_\lambda P' H p_k. \quad (10)$$

Denoting by $D(\zeta)$ the determinant of n th order

$$D(\zeta) = \det. \{H w_k + H p_k - \zeta p_k, p_i\}, \quad (11)$$

We have

THEOREM IV. *The function $\Psi(\zeta) = \frac{D(\zeta)}{\Gamma\{p_k\}}$ is given by*

$$\Psi(\zeta) = \frac{1}{\Phi(\zeta)} = (-\zeta)^n \prod_{k=0}^{\infty} \frac{(\zeta - \lambda_k)}{(\zeta - \lambda'_k)} \prod_{k=0}^{\infty} \frac{(\zeta - \mu_k)}{(\zeta - \mu'_k)}. \quad (12)$$

5. The proof of theorems III and IV is based on a few lemmas. We use first

LEMMA 1. *The function $\Phi(\zeta)$ of (9) does not depend on the choice of vectors $\{p_k\}$ generating $\mathcal{L} \ominus \mathcal{L}'$.*

This lemma allows us to limit ourselves to the case of orthonormal vectors p_k . In writing

$$\Phi(\zeta) = \Phi_{\mathcal{L}', \mathcal{L}}(\zeta), \quad (13)$$

we can state

LEMMA 2. *If $\mathcal{L}' \subset \mathcal{L}'' \subset \mathcal{L}$, then $\Phi_{\mathcal{L}', \mathcal{L}''} \Phi_{\mathcal{L}'', \mathcal{L}} = \Phi_{\mathcal{L}', \mathcal{L}}$.*

This lemma is proved firstly in the case when $\mathcal{L} \ominus \mathcal{L}''$ is one dimensional and the general case follows immediately.

Following these two lemmas, the proof of Theorem I is reduced to the case when $\mathcal{L} \ominus \mathcal{L}'$ is one dimensional. In this case we have only one vector, p_1 , $\|p_1\| = 1$, and using the spectral representation of the resolvent operator, we obtain

$$\Phi(\zeta) = \sum_{k=0}^{\infty} \frac{|\alpha_k^+|^2}{\lambda_k - \zeta} + \sum_{k=0}^{\infty} \frac{|\alpha_k^-|^2}{\mu_k - \zeta} - \frac{1}{\zeta} \sum_{k=1}^{\infty} |\alpha_k^0|^2, \quad (14)$$

where $\alpha_k^+ = (p_1, u_k^+)$, $\alpha_k^- = (p_1, u_k^-)$, $\alpha_k^0 = (p_1, u_k^0)$.

We use then the following lemma on analytic functions.

LEMMA 3. Any meromorphic function of the form

$$\Phi(\zeta) = \frac{-\gamma}{\zeta} + \sum_{k=0}^{\infty} \frac{\gamma_k^+}{\omega_k^+ - \zeta} + \sum_{k=0}^{\infty} \frac{\gamma_k^-}{\omega_k^- - \zeta}, \quad (15)$$

where $\gamma_k^+ > 0$, $\gamma_k^- > 0$, $\gamma \geq 0$, $\gamma + \sum (\gamma_k^+ + \gamma_k^-) = 1$, $\omega_0^+ > \omega_1^+ > \dots \rightarrow 0$, $\omega_0^- < \omega_1^- < \dots \rightarrow 0$, is representable in the form

$$\Phi(\zeta) = \frac{-1}{\zeta} \prod_0 \frac{\zeta - \tau_k^+}{\zeta - \omega_k^+} \prod_0 \frac{\zeta - \tau_k^-}{\zeta - \omega_k^-}, \quad (16)$$

where $\omega_k^+ > \tau_k^+ > \omega_{k+1}^+$, $\omega_k^- < \tau_k^- < \omega_{k+1}^-$. Inversely, every function of the form (16) is representable in the form (15). Both representations are unique, the τ_k^+ and τ_k^- are the zeros of Φ and we have the formulas:

$$\begin{aligned} \gamma &= \prod \frac{\tau_k^+}{\omega_k^+} \prod \frac{\tau_k^-}{\omega_k^-}, \\ \gamma_l^+ &= \frac{\omega_l^+ - \tau_l^+}{\omega_l^+} \prod_{k \neq l} \frac{\omega_l^+ - \tau_k^+}{\omega_l^+ - \omega_k^+} \prod \frac{\omega_l^+ - \tau_k^-}{\omega_l^+ - \omega_k^-}, \\ \gamma_l^- &= \frac{\omega_l^- - \tau_l^-}{\omega_l^-} \prod \frac{\omega_l^- - \tau_k^-}{\omega_l^- - \omega_k^-} \prod_{k \neq l} \frac{\omega_l^- - \tau_k^+}{\omega_l^- - \omega_k^+}. \end{aligned}$$

This lemma gives us the representation (16), similar to (9) for the case of $n = 1$. In order to obtain the exact formula (9) from (16) (for $n = 1$) we use the following lemma.

LEMMA 4. In the case of a one-dimensional $\mathcal{L} \ominus \mathcal{L}'$, for any positive (or negative) number ζ' , the difference of multiplicity of ζ' in $\{\lambda'_k\}$ and $\{\lambda_k\}$ (or $\{\mu'_k\}$ and $\{\mu_k\}$) is equal to $-1, 0$ or 1 , depending on ζ' being a pole, an ordinary point or a zero of $\Phi(\zeta)$.

The term "multiplicity of ζ' " in a monotonic sequence $\{\rho_k\}$ means the number of times ζ' appears in this sequence.

After having proved Theorem III, we pass to Theorem IV. It is sufficient to prove here that $\Psi = 1/\Phi$. This is achieved by comparing the expressions of the determinants $W(\zeta)$ and $D(\zeta)$.

6. The problem in which we are interested is the calculation of the eigenvalues of the operator H in a given subspace \mathcal{L} . On the basis of the preceding theorems, we will develop two methods for the computation of these eigenvalues.

A. *Weinstein's Method.*—Suppose that for some subspace $\mathcal{L}^{(0)} \supset \mathcal{L}$ we know the eigenvalues and eigenvectors of H in $\mathcal{L}^{(0)}$, and suppose further, that for a sequence of vectors, p_1, p_2, \dots of the subspace $\mathcal{L}^{(0)} \ominus \mathcal{L}$, we know the vectors $v_k(\lambda) = R_\lambda^{(0)} p_k$. We consider then the subspace $\mathcal{L}^{(n)} =$

$\mathcal{L}^{(0)} \ominus \mathcal{O}^{(n)}$ where $\mathcal{O}^{(n)}$ is the n -dimensional subspace generated by the n first vectors p_k .

Our assumptions allow us to form explicitly the function $\Phi_n(\xi) \equiv \Phi_{\mathcal{L}^{(n)}, \mathcal{L}^{(0)}}(\xi)$, and by Theorem III, knowing the eigenvalues $\{\lambda_k^{(0)}\}$ and $\{\mu_k^{(0)}\}$ we can find the eigenvalues $\{\lambda_k^{(n)}\}$ and $\{\mu_k^{(n)}\}$.

By Theorem A, we have

$$\lambda_k^{(n)} \geq \lambda_k^{(n+1)} \geq \lambda_k, \quad \mu_k^{(n)} \leq \mu_k^{(n+1)} \leq \mu_k.$$

If the sequence $\{p_k\}$ is complete in the subspace $\mathcal{L}^{(0)} \ominus \mathcal{L}$, the subspaces $\mathcal{L}^{(n)} \searrow \mathcal{L}$ and by Corollary II', $\lambda_k^{(n)} \searrow \lambda_k$ and $\mu_k^{(n)} \nearrow \mu_k$.

Remark: We can give here a practical rule for establishing the sequence $\{\lambda_k^{(n)}\}$ (for the $\mu_k^{(n)}$ there is a similar rule). Consider the sequence of positive poles $\omega_1 \geq \omega_2 \geq \dots$ and of positive zeros $\tau_1 \geq \tau_2 \geq \dots$ of $\Phi_n(\xi)$. $\{\omega_k\}$ is a subsequence of the sequence $\{\lambda_k^{(0)}\}$, i.e., it is of the form $\{\lambda_{k_l}^{(0)}\}$, $l = 1, 2, \dots$. Consider then the complementary terms $\lambda_{k'}^{(0)}$ for all $k' \neq k_l$ and order all these terms together with the zeros τ_k in one non-increasing sequence. The obtained sequence will be $\{\lambda_k^{(n)}\}$.

B. Rayleigh-Ritz Method.—Suppose now that for some subspace $\mathcal{L}^{(0)} \subset \mathcal{L}$ we know completely the eigenvalues, eigenvectors, resolvent operator and the part of H in $\mathcal{L}^{(0)}$.

Consider then a sequence of vectors $\{p_k\}$ contained in $\mathcal{L} \ominus \mathcal{L}^{(0)}$. We introduce the increasing sequence of subspaces $\mathcal{L}^{(n)} = \mathcal{L}^{(0)} \oplus \mathcal{O}^{(n)}$, where $\mathcal{O}^{(n)}$ is the n -dimensional subspace generated by the n first p_k . By our assumptions we can calculate the function $\Psi_n(\xi) \equiv \Psi_{\mathcal{L}^{(n)}, \mathcal{L}^{(0)}}(\xi)$, and knowing the eigenvalues of H in $\mathcal{L}^{(0)}$, we obtain, by Theorem IV, the eigenvalues of H in $\mathcal{L}^{(n)}$. By Theorem A, we have

$$\lambda_k^{(n)} \leq \lambda_k^{(n+1)} \leq \lambda_k, \quad \mu_k^{(n)} \geq \mu_k^{(n+1)} \geq \mu_k.$$

If the p_k form a complete system in $\mathcal{L} \ominus \mathcal{L}^{(0)}$, we get $\lambda_k^{(n)} \nearrow \lambda_k$, and $\mu_k^{(n)} \searrow \mu_k$. This method will be called the *generalized Rayleigh-Ritz method*. The *ordinary Rayleigh-Ritz method* corresponds to the case when we take $\mathcal{L}^{(0)} = (0)$, i.e., the subspace containing only the zero vector. It is clear that in this case all our assumptions will be fulfilled so that the ordinary Rayleigh-Ritz method may always be applied.

7. It is clear now that the two methods are complementary in character. The Rayleigh-Ritz method gives lower bounds for λ_k , upper bounds for μ_k , whereas the Weinstein method gives upper bounds for λ_k , lower bounds for μ_k . The combination of the two (even by the use of a small number of p_k) may lead to quite good approximations of the eigenvalues of H in \mathcal{L} . A method may be developed for evaluating the errors made in taking $\lambda_k^{(n)}$ instead of λ_k . In the case of a definite positive operator we can use, for instance, Corollary I' of § 3 and obtain inequalities of the type $|\lambda_k^{(n)} - \lambda_k| \leq \lambda_0^{(n)}$, where $\lambda_k^{(n)}$ are the eigenvalues of H in the subspace $\mathcal{L}^{(n)} \ominus \mathcal{L}$.

for the Weinstein method and $\mathfrak{L} \ominus \mathfrak{L}^{(n)}$ for the Rayleigh-Ritz method, respectively.

8. Lemma 3 of § 5 allows the complete solution of the following problem. Given the eigenvalues $\{\lambda_k\}$ and $\{\mu_k\}$ of H in \mathfrak{L} , find the characteristic properties of the eigenvalues $\{\lambda'_k\}$ and $\{\mu'_k\}$ of H in a subspace $\mathfrak{L}' \subset \mathfrak{L}$.

We will mention here only the solution of this problem in the case of a positive definite H . We have then only the sequence $\lambda_0 \geq \lambda_1 \geq \lambda_2 \geq \dots \rightarrow 0$. For $\lambda'_0 \geq \lambda'_1 \geq \lambda'_2 \geq \dots \rightarrow 0$ to be the sequence of the eigenvalues of H in a subspace $\mathfrak{L}' \subset \mathfrak{L}$, it is necessary and sufficient that $\lambda'_k \leq \lambda_k$ for every k and that for some $h = 1, 2, \dots$, the following condition be fulfilled

$$\prod_{n=0}^{\infty} \frac{\lambda'_{n,h}}{\lambda_n} = 0, \quad (17_h)$$

where $\lambda'_{n,h} = \max. (\lambda'_n, \lambda_{n+h})$.

It should be remarked that for two sequences $\{\lambda_k\}$ and $\{\lambda'_k\} \searrow 0$, satisfying the condition $\lambda'_k \leq \lambda_k$, the conditions (17_h) , for any values of h , are always equivalent. From (17_h) follows the condition

$$\prod_0^{\infty} \frac{\lambda'_m}{\lambda_m} = 0. \quad (18)$$

We can get more precise information about λ'_k if we require them to be the eigenvalues of H in a subspace $\mathfrak{L}' \subset \mathfrak{L}$ such that $\mathfrak{L} \ominus \mathfrak{L}'$ be n -dimensional, $n < \infty$. Then the necessary and sufficient conditions are $\lambda_k \geq \lambda'_k \geq \lambda_{k+n}$, together with the condition (18).

* The results of the present and the following papers were obtained by the author in 1943 as far as the positive definite operators are concerned. A résumé of these results was circulated in a few copies among a number of mathematicians, but were never published. The extension to indefinite operators is much more recent.

¹ Ritz, W., *J. Reine Angew. Math.*, **135**, 1 (1908); Courant, R. *Bull. Am. Math. Soc.* **49**, 1 (1943).

² Weinstein, A., *Memorial des Sciences Math.*, **88** (1937).

³ Courant-Hilbert, *Methoden der Math. Physik*, Vol. I, 2nd ed., Berlin, pp. 26-29, 112-113, 351.

⁴ This kind of convention was first introduced by H. Weyl, *Math. Ann.*, **71**, 443 (footnote), 1912.

⁵ These theorems are known especially for differential operators, cf. Courant-Hilbert, *loc. cit.*, p. 353. These operators in usual cases are the inverses of completely continuous integral operators and consequently the inequalities in the theorems have to be inverted.

EXPONENTIAL TRANSFORMS AND APPELL POLYNOMIALS

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1. By an exponential transform we mean an integral of the form

$$\int_0^\infty f(t)e^{\alpha t} dt,$$

where α is a complex number of absolute value one. The choice $\alpha = -1$ gives the usual form of the Laplace transform. The bilateral Laplace transform is the sum of two exponential transforms with $\alpha = -1$ and $\alpha = 1$; the Fourier transform is the sum of two exponential transforms with $\alpha = i$ and $\alpha = -i$. A bilateral Laplace transform converges, in general, in a vertical strip of the complex plane; a Fourier transform converges, in general, in a horizontal strip; but not every function analytic in a vertical or horizontal strip is represented by one of these transforms. The sum of a bilateral Laplace and a Fourier transform converges, in general, in a rectangle. This fact suggests the investigation of what analytic functions are representable by such sums of transforms or, in general, by a sum of $n \geq 3$ exponential transforms in which the α 's do not lie on a straight line. The convergence region of such a sum is in general a bounded convex polygon, the intersection of n half planes; it turns out that conversely every function analytic in a convex n -gon can be represented as a sum of n exponential transforms. In particular, every analytic function has (infinitely many) representations as a sum of three exponential transforms.

The proof of this result is a simple application of Cauchy's integral formula. Let $F(z)$ be analytic in a closed bounded n -gon P whose sides are line segments L_k and represent $F(z)$ in the interior of P by Cauchy's formula:

$$F(z) = \sum_{k=1}^n (2\pi i)^{-1} \int_{L_k} (t-z)^{-1} F(t) dt.$$

Let P be the intersection of the half planes $\Re(te^{i\theta_k}) < c_k$, so that the equation of L_k is $\Re(te^{i\theta_k}) = c_k$, and let $\alpha_k = -e^{i\theta_k}$. If $\Re(-\alpha_k z) > c_k$, we have $\Re(\alpha_k(t-z)) > 0$ for t on L_k and

$$\alpha_k \int_0^\infty e^{-\alpha_k(t-z)u} du = (t-z)^{-1};$$

since $|e^{-\alpha_k(t-z)}| = e^{c_k - \Re(-\alpha_k z)}$, the integral converges absolutely and uniformly (in t) for t on L_k . Hence

$$\begin{aligned} (2\pi i)^{-1} \int_{L_k} (t-z)^{-1} F(t) dt &= \int_0^\infty e^{\alpha_k z u} du \int_{L_k} (2\pi i)^{-1} \alpha_k F(t) e^{-\alpha_k t u} dt \\ &= \int_0^\infty e^{\alpha_k z u} f_k(u) du, \end{aligned}$$

say. Thus

$$F(z) = \sum_{k=1}^n \int_0^\infty e^{\alpha_k z u} f_k(u) du$$

in the intersection of all the half planes $\Re(\alpha_k z) < c_k$, that is, in P .

2. The applications of the result of § 1 to Appell polynomials arise as follows. An Appell set $\{p_n(z)\}$ is defined by the formal expansion

$$A(t)e^{zt} = \sum_{n=0}^{\infty} t^n p_n(z),$$

where $A(t)$ stands for the formal power series $\sum_{n=0}^{\infty} a_n t^n$ and is called the generating function of the set $\{p_n(z)\}$. The class of functions representable by series $\sum_{n=0}^{\infty} c_n p_n(z)$ depends on properties of $A(t)$; it was found by Sheffer¹ that the appropriate class of generating functions $A(t)$ for representing analytic functions in general is the class of entire functions of exponential type. Sheffer proved that, if $A(t)$ belongs to this class, every function analytic in a sufficiently large circle with center at 0 has an Appell expansion representing it in some neighborhood of 0; if the type of $A(t)$ is zero, the expansion represents the function in its circle of regularity with center at 0, just like a power series (which corresponds to $A(t) \equiv 1$). Sheffer's results were obtained from a study of linear functional equations associated with the Appell set.

Suppose now that $F(z)$ is analytic at $z = 0$ and that we represent $F(z)$ as a sum

$$F(z) = \sum_{k=1}^m \int_0^\infty f_k(t) e^{\alpha_k z t} dt \quad (1)$$

of exponential transforms. We have

$$e^{\alpha_k z t} = \{1/A(t)\} \sum_{n=0}^{\infty} t_n \alpha_k^n p_n(z),$$

and if we substitute these expansions into (1) we obtain, formally,

$$F(z) = \sum_{n=0}^{\infty} p_n(z) \sum_{k=1}^m \alpha_k^n \int_0^\infty \{f_k(t)/A(\alpha_k t)\} t^n dt,$$

a series in the polynomials $p_k(z)$. To justify the formal process for some domain of values of z we need sufficient information about the behavior of $1/A(t)$. In general it is necessary to alter the path of integration in each exponential transform in order to avoid the zeros of $A(t)$. The more precise our knowledge of the behavior of $1/A(t)$, the sharper our expansion theorem will be. It is possible in this way to prove Sheffer's general

theorem and to obtain more precise results for special classes of generating functions. An advantage of this approach is that, since the theory of the associated functional equations is not used, the Appell expansion becomes available as a tool for studying the functional equations. Furthermore, the method works equally well for a large class of the more general polynomial sets defined by

$$A(t)e^{zt} = \sum_{n=0}^{\infty} \{B(t)\}^n p_n(z),$$

where $B(0) = 0$, $B'(0) = 1$ (Sheffer's "sets of type 0") and the results so obtained could also be applied to functional equations.

To carry out the discussion outlined here requires more information about the reciprocal of an entire function than seems to be available in the literature. The details will be given elsewhere.

¹ Sheffer, I. M., "Some Applications of Certain Polynomial Classes," *Bull. Am. Math. Soc.*, **47**, 885-898 (1941); further references are given there.

NOTES ON INTEGRATION, III

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As part of our theory of general integration begun in earlier notes,¹ we shall now establish general forms of the Fubini theorem and its extensions. Since the Fubini theorem deals with multiple and iterated integration, our notation must be modified so that at least three general integrations, attached to as many different domains, can be handled simultaneously without confusion. For our present purposes it suffices to indicate clearly the particular domain to which is attached each mathematical object under consideration. Thus the different families of functions which have to be considered on a given domain X will be denoted as $\mathfrak{E}(X)$, $\mathfrak{G}(X)$, $\mathfrak{L}(X)$, and so on. Operations on such functions will be denoted, in a slightly different fashion, as E_x , N_x , L_x , and so on; and the results of applying such operations to a particular function f will be denoted as $E_x f(x)$, $N_x f(x)$, $L_x f(x)$, and so on.² Furthermore it will be convenient to follow the common practice in shortening the precise phrase "the function f whose value at x is $f(x)$ " to the handier phrase "the function $f(x)$."

Let $Z = X \times Y$ be the Cartesian product of X and Y —that is, the totality of pairs $z = (x, y)$ where $x \in X$ and $y \in Y$. Let E_x and E_y be elementary integrals defined for the respective families $\mathfrak{E}(X)$ and $\mathfrak{E}(Y)$ of

elementary functions. We then designate by $\mathfrak{E}(X) * \mathfrak{E}(Y)$ the totality of real functions $f(z) = f(x, y)$ with the following properties: for fixed x , the function $f(x, y)$ is in $\mathfrak{E}(Y)$; and the integral $E_y f(x, y)$ is a function in $\mathfrak{E}(X)$. This family is obviously linear but is not guaranteed to contain $|f|$ whenever it contains f ; it fails in this respect to conform to the requirements imposed upon a family of elementary functions. Further, we designate by $E_x * E_y$ the operation which takes any function f with the above properties into the real number $E_x E_y f(x, y)$. This is a positive linear operation which even satisfies the condition I (2) under the hypothesis that $|f|$ and $|f_n|$ are in $\mathfrak{E}(X) * \mathfrak{E}(Y)$: indeed, if $|f(x, y)| \leq \sum_{n=1}^{\infty} |f_n(x, y)|$, application of I (2) to E_y yields $E_y |f(x, y)| \leq \sum_{n=1}^{\infty} E_y |f_n(x, y)|$ for each x and then application of I (2) to E_x yields $E_x E_y |f(x, y)| \leq \sum_{n=1}^{\infty} E_x E_y |f_n(x, y)|$.

If $\mathfrak{E}(Z)$ is a linear subfamily of $\mathfrak{E}(X) * \mathfrak{E}(Y)$ which contains $|f|$ together with f and if E_z is the contraction of $E_x * E_y$ to $\mathfrak{E}(Z)$, we therefore see that $\mathfrak{E}(Z)$ and E_z satisfy I (1) and I (2); and hence that E_z can be regarded as an elementary integral and $\mathfrak{E}(Z)$ as the family of elementary functions over which it is defined. There are many important examples where $\mathfrak{E}(Z)$ can be specified so as to contain all the functions $h(z) = h(x, y) = f(x)g(y)$ where $f \in \mathfrak{E}(X)$ and $g \in \mathfrak{E}(Y)$, such functions h obviously being members of $\mathfrak{E}(X) * \mathfrak{E}(Y)$. Frequently E_z is given directly without reference to E_x and E_y , and has to be identified as a contraction of $E_x * E_y$ —in other words, the relation $E_z \subset E_x * E_y$ has to be proved as a theorem. Since illustrations of these remarks are well known we shall not go into greater detail here. We must, however, call particular attention to the fact that in general we will have $\mathfrak{E}(X) * \mathfrak{E}(Y) \neq \mathfrak{E}(Y) * \mathfrak{E}(X)$ and $E_x * E_y \neq E_y * E_x$. This lack of symmetry may well extend so far that for some functions f we have $E_x E_y f(x, y) \neq E_y E_x f(x, y)$; but, of course, the functions $h(z) = h(x, y) = f(x)g(y)$ where $f \in \mathfrak{E}(X)$ and $g \in \mathfrak{E}(Y)$ are not among them. On the other hand there are many familiar and important cases where $\mathfrak{E}(Z)$ can be specified so that $E_x * E_y$ and $E_y * E_x$ have identical contractions to $\mathfrak{E}(Z)$. In such a case we have $E_z f(z) = E_x E_y f(x, y) = E_y E_x f(x, y)$ and the relation of E_z to E_x and E_y involves the latter in a symmetric manner.

Turning now to the general integrations L_x and L_y associated with E_x and E_y , respectively, we shall introduce an operation $L_x * L_y$ analogous to the operation $E_x * E_y$ of the preceding paragraph. First we designate by $\mathfrak{L}(X) * \mathfrak{L}(Y)$ the family of all extended-real functions $f(z) = f(x, y)$ with the following properties: for each x outside a fixed null subset X_0 of X the function $f(x, y)$ is in $\mathfrak{L}(Y)$; and the integral $L_y f(x, y)$ is a function defined outside X_0 and coinciding there with a function g in $\mathfrak{L}(X)$. We then define $L_x * L_y$ as the operation which takes such a function f into the

real number $L_x g(x)$ where $g(x) = L_y f(x, y)$ for x outside X_0 and $g \in \mathfrak{F}(X)$, observing that this number is unaffected by the ambiguity in the determination of g . As we shall see below, the generalized Fubini theorem is conveniently expressed in terms of the operation $L_x * L_y$.

As a preliminary to the statement and proof of the generalized Fubini theorem, we first make the following observation:

- (1) *if the elementary integrations E_x , E_y , and E_z satisfy the relation $E_z \subset E_x * E_y$, then the corresponding operations N_x , N_y , N_z are such that $N_z f(z) \geq N_x N_y f(x, y)$ for every f in $\mathfrak{G}(Z)$.*

The proof is simple. Since we have nothing to prove unless $N_z f(z) < +\infty$, we assume the latter relation. We can then choose f_n in $\mathfrak{G}(Z)$ so that $|f| \leq \sum_{n=1}^{\infty} |f_n|$ and $\sum_{n=1}^{\infty} E_x E_y |f_n(x, y)| = \sum_{n=1}^{\infty} E_z |f_n(z)| \leq N_z f(z) + \epsilon$ for any given $\epsilon > 0$. On the other hand we have $N_y f(x, y) \leq \sum_{n=1}^{\infty} N_y f_n(x, y) = \sum_{n=1}^{\infty} E_y |f_n(x, y)|$ by I (7) and I (9); and hence $N_x N_y f(x, y) \leq \sum_{n=1}^{\infty} N_x E_y |f_n(x, y)| = \sum_{n=1}^{\infty} E_x E_y |f_n(x, y)| \leq N_z f(z) + \epsilon$ by I (7), I (9), and the above. Since $\epsilon > 0$ is arbitrary, the theorem is established.

We now state the first part of the generalized theorem of Fubini:

- (2) (Fubini) *if the elementary integrations E_x , E_y , and E_z satisfy the relation $E_z \subset E_x * E_y$, then the corresponding general integrations satisfy the relation $L_z \subset L_x * L_y$ —in other words the general integral $L_z f(z)$ can be evaluated as the iterated integral $L_x L_y f(x, y)$ in the sense made precise above.*

With the help of (1) the proof offers little difficulty. If f is in $\mathfrak{F}(Z)$ we can find f_n in $\mathfrak{G}(Z)$ so that $N_z(f(z) - f_n(z)) \leq 2^{-n}$. The positive-term series $\sum_{n=1}^{\infty} N_y(f(x, y) - f_n(x, y))$ has sum $h(x)$ in $\mathfrak{G}(X)$; and the relation $N_x h(x) \leq \sum_{n=1}^{\infty} N_x N_y(f(x, y) - f_n(x, y)) \leq \sum_{n=1}^{\infty} N_z(f(z) - f_n(z)) \leq \sum_{n=1}^{\infty} 2^{-n} = 1$ shows that $h \in \mathfrak{F}(X)$ and hence that h is finite except on a null set X_0 . If x is outside X_0 we therefore have $\lim_{n \rightarrow \infty} N_y(f(x, y) - f_n(x, y)) = 0$ and hence $f(x, y) \in \mathfrak{F}(Y)$. We let $g(x)$ be any function in $\mathfrak{G}(X)$ which is equal to $L_y f(x, y)$ outside X_0 . Since $|g(x) - E_y f_n(x, y)| = |L_y f(x, y) - E_y f_n(x, y)| = |L_y(f(x, y) - f_n(x, y))| \leq L_y |f(x, y) - f_n(x, y)| = N_y(f(x, y) - f_n(x, y))$ almost everywhere, we see that $N_x(g(x) - E_y f_n(x, y)) \leq N_x N_y(f(x, y) - f_n(x, y)) \leq N_z(f(z) - f_n(z)) \leq 2^{-n}$. Hence $g(x)$ is in $\mathfrak{F}(X)$ and $L_x g(x) = \lim_{n \rightarrow \infty} E_x E_y f_n(x, y) = \lim_{n \rightarrow \infty} E_z f_n(z) = L_z f(z)$ in accordance with the definitions of L_x and L_z . The remainder of the generalized theorem of Fubini is the following partial converse of (2):

- (3) (Fubini) if the elementary integrations E_x , E_y , and E_z satisfy the relation $E_z \subset E_x * E_y$, if $f \in \mathfrak{M}(Z)$, and if there exist functions f_n in $\mathfrak{F}(Z)$ such that $|f| \leq \sum_{n=1}^{\infty} |f_n|$, then $|f| \in \mathfrak{L}(X) * \mathfrak{L}(Y)$ implies $f \in \mathfrak{L}(Z)$.

In accordance with II (14) we may suppose without loss of generality that $f_n \in \mathfrak{L}(Z)$. The function $g_n = \min(|f|, |f_1| + \dots + |f_n|)$ is also in $\mathfrak{L}(Z)$ in accordance with II (6), II (7). Since $0 \leq g_n \leq |f|$ we see that $N_y g_n(x, y) \leq N_y f(x, y)$ and $N_z N_y g_n(x, y) \leq N_z N_y f(x, y)$. The fact that $|f|$ is in $\mathfrak{L}(X) * \mathfrak{L}(Y)$ shows that $N_y f(x, y) = L_y |f(x, y)|$ for almost all x , and also that $N_y f(x, y)$ differs only on a null set from an integrable function $g(x)$ and is therefore integrable itself. Thus $N_z N_y f(x, y) = L_z g(x) < +\infty$. On the other hand the inequalities $g_n \leq g_{n+1}$ and $|f| \leq \sum_{n=1}^{\infty} |f_n|$ show that $\{g_n\}$ is a monotonely increasing sequence which converges to $|f|$. Hence $N_z f(z) = \lim_{n \rightarrow \infty} L_z g_n(z)$. Application of (2) to $g_n \in \mathfrak{L}(Z)$ yields $L_z g_n(z) = N_z N_y g_n(x, y) \leq N_z N_y f(x, y)$. Thus $N_z f(z) \leq N_z N_y f(x, y) < +\infty$ so that $f \in \mathfrak{F}(Z)$. Since it was assumed that $f \in \mathfrak{M}(Z)$, we conclude by II (11) that $f \in \mathfrak{L}(Z)$. It is well known that the hypotheses of (3) cannot be weakened in any essential respect. Membership in $\mathfrak{L}(X) * \mathfrak{L}(Y)$ does not imply membership in $\mathfrak{M}(Z)$, so that some hypothesis concerning the measurability of f is needed in order to guarantee that $f \in \mathfrak{L}(Z)$. The need for the condition that $|f| \leq \sum_{n=1}^{\infty} |f_n|$ for appropriate f_n in $\mathfrak{F}(Z)$ is illustrated by a simple example of Saks.³ This condition is automatically satisfied in many of the common instances of our general theory. In particular if $1 \in \mathfrak{L}(Z)$ we can always take $f_n = 1$.

Our version of the Fubini theorem can be applied directly to a situation in the theory of locally compact topological groups.⁴ Let Z be such a group, Y one of its closed subgroups and X the homogeneous space of left cosets of Y . Selecting from each coset x a fixed element z_x we see that the equation $z = z_x y$ defines a one-to-one correspondence between Z and $X \times Y$, which can therefore be identified as abstract sets during the remainder of the discussion. We let $\mathfrak{C}(X)$, $\mathfrak{C}(Y)$, $\mathfrak{C}(Z)$ be the families of continuous real functions with compact nuclei on the respective spaces X , Y , Z . Three elementary integrations E_x , E_y , E_z defined over $\mathfrak{C}(X)$, $\mathfrak{C}(Y)$, $\mathfrak{C}(Z)$, respectively, will be said to form an admissible triple if E_y is left-invariant, E_x and E_z are relatively left-invariant and $E_z f(z) = E_x E_y f(z' y)$ for all f in $\mathfrak{C}(Z)$. It is implicit in this definition that the integral $E_y f(z' y)$ is constant on each coset x and can therefore be considered as a function on X which is, in fact, a member of $\mathfrak{C}(X)$. We observe now that E_x , E_y , E_z constitute an admissible triple if and only if, in addition to

enjoying the required invariance properties, they satisfy the relation $E_z \subset E_x * E_y$. To verify this we need only note that for any f in $\mathfrak{E}(Z)$ we have $f(z) = f(z_x y) = f(x, y)$ and hence $E_y f(x, y) = E_y f(z_x y) = E_y f(z' y)$ for all z' in the coset x : for we then have $E_y f(x, y) \in \mathfrak{E}(X)$ and the conditions $E_z f(z) = E_x E_y f(z' y)$ and $E_z \subset E_x * E_y$ are therefore equivalent. The group-theoretic conditions for the existence of admissible triples are discussed by A. Weil.⁴ Here we direct attention to the fact that when $\{E_x, E_y, E_z\}$ is an admissible triple, the associated general integrations L_x, L_y, L_z (which enjoy corresponding invariance properties) must satisfy the relation $L_z \subset L_x * L_y$ in accordance with (2) above.⁵

We turn finally to an extension of the Fubini theorem, due originally to Jessen in a particular case.⁶ With each element λ of a fixed infinite class Λ let there be associated a non-void set X_λ . We denote by X_Λ the Cartesian product of those X_λ with $\lambda \in \Lambda$. For each non-void finite part B of Λ let there be given an elementary integration E_{x_B} defined for a family $\mathfrak{E}(X_B)$ of elementary functions on X_B . Let it be assumed that the following conditions hold:

- (4) the constant function everywhere equal to 1 on X_B is in $\mathfrak{E}(X_B)$ and its elementary integral is 1;
- (5) if Γ and Δ constitute a partition of B and if $g \in \mathfrak{E}(X_\Gamma)$, then the function f defined by $f(x_B) = f(x_\Gamma, x_\Delta) = g(x_\Gamma)$ is in $\mathfrak{E}(X_B)$;
- (6) if Γ and Δ constitute a partition of B , then $E_{x_B} \subset E_{x_\Gamma} * E_{x_\Delta}$.

For an arbitrary infinite part A of Λ we can now define⁷ $\mathfrak{E}(X_A)$ and E_{x_A} satisfying I (1): we take $\mathfrak{E}(X_A)$ to be the family of those functions f on X_A such that for some partition of A into a finite set B and its complement Γ and for some g in $\mathfrak{E}(X_B)$ the relation $f(x_A) = f(x_B, x_\Gamma) = g(x_B)$ is valid; and for each such f we put $E_{x_A} f(x_A) = E_{x_B} f(x_B, x_\Gamma) = E_{x_B} g(x_B)$. We assume finally that I (2) holds for $\mathfrak{E}(X_A)$ and E_{x_A} . It is then evident that I (2) must also hold for $\mathfrak{E}(X_A)$ and E_{x_A} , whatever the infinite set $A \subset \Lambda$. As an instance where all our assumptions can easily be verified, we cite one equivalent to that given by Jessen:⁶ we take X_λ to be the unit interval, $0 \leq x_\lambda \leq 1$; $\mathfrak{E}(X_B)$ to be the family of all continuous real functions on the hypercube X_B ; and E_{x_B} to be the Riemann integral. In the general case a rather simple analysis, which we shall not repeat, shows that

- (7) in (4), (5) and (6) the finite set B can be replaced by an arbitrary infinite set $A \subset \Lambda$.

We now direct attention to a remarkable property of the general integration associated with E_{x_A} , namely:

- (8) if $f \in \mathfrak{E}_p(X_A)$ then there exist a partition of A into sets Γ and Δ , where Γ is countable, and a function g in $\mathfrak{E}_p(X_\Gamma)$ such that $f(x_A) = f(x_\Gamma, x_\Delta) = g(x_\Gamma)$ for almost all x_A .

Because of the mapping of $\mathfrak{L}_p(X_A)$ onto $\mathfrak{L}(X_A)$ discussed in II, it suffices to treat the case $p = 1$. There we determine functions f_n in $\mathfrak{L}(X_A)$ such that $N(f - f_n) \leq 2^{-n}$. As in the proof of I (10), we find that f and $\limsup_{n \rightarrow \infty} f_n = h$ differ only on a null set. In view of the definition of $\mathfrak{L}(X_A)$ there exists a countable set Γ such that for every n the function $f_n(x_A) = f_n(x_\Gamma, x_\Delta)$ is constant with respect to x_Δ . Hence $h(x_A) = h(x_\Gamma, x_\Delta)$ has the same property. The function $g(x_\Gamma) = h(x_\Gamma, x_\Delta)$ is then in $\mathfrak{L}(X_\Gamma)$, since (6) and (7) above permit the application of (2). Jessen's most interesting results concern the case where A is countable; but (8) shows that in handling a finite or countably infinite family of functions in $\mathfrak{L}_p(X_A)$ there is no loss of generality in restricting attention to this case. We suppose therefore that A is the class of positive integers, and establish the following result:

- (9) (Jessen) if $f \in \mathfrak{L}_p(X_A)$, $B = \{\alpha; \alpha \leq n\}$, and $\Gamma = \{\alpha; \alpha > n\}$, then there exist functions g_n, h_n in $\mathfrak{L}_p(X_A)$ defined for almost all X_A by the relations $g_n(x_A) = g_n(x_B, x_\Gamma) = L_{x_B}f(x_B, x_\Gamma)$, $h_n(x_A) = h_n(x_B, x_\Gamma) = L_{x_\Gamma}f(x_B, x_\Gamma)$; and in $\mathfrak{L}_p(X_A)$ the convergence relations $g_n \rightarrow L_{x_B}f(x_A)$, $h_n \rightarrow f$ are valid.

Only h_n will be discussed in detail, as g_n can be treated in much the same way. Since $1 \in \mathfrak{L}(X_\Delta)$ for any $\Delta \subset A$, we see that $\mathfrak{L}_p(X_\Delta) \subset \mathfrak{L}(X_\Delta)$ and that all the techniques developed in II are available to us here. The Fubini theorem is also available to us. Thus we can use this theorem to infer first that h_n exists as a member of $\mathfrak{L}(X_A)$. If f is in $\mathfrak{L}_p(X_A)$, the inequality $N_{x_A}|h_n(x_A)|^p = N_{x_B}|L_{x_\Gamma}f(x_B, x_\Gamma)|^p \leq N_{x_B}L_{x_\Gamma}|f(x_B, x_\Gamma)|^p = L_{x_B}L_{x_\Gamma}|f(x_B, x_\Gamma)|^p = L_{x_A}|f(x_A)|^p = N_{x_A}|f(x_A)|^p < +\infty$ shows that h_n is also in $\mathfrak{L}_p(X_A)$. If f is in $\mathfrak{L}_p(X_A)$ we can find for any $\epsilon > 0$ a function \tilde{f} in $\mathfrak{L}_p(X_A)$ such that $\tilde{f}(x_A) = \tilde{f}(x_B, x_\Gamma)$ is constant with respect to x_Γ for some choice of $B = \{\alpha; \alpha \leq m\}$ while $N_p(f - \tilde{f}) \leq \frac{1}{2}\epsilon$. Using the notations of II, we choose \tilde{f} so that $\tilde{g} = \Phi(\tilde{f}) \in \mathfrak{L}(X_A) \subset \mathfrak{L}(X_\Delta)$ and $N(\Phi(f) - \tilde{g}) \leq \delta$. Since $B = \{\alpha; \alpha \leq m\}$ can be chosen so that $\tilde{g}(x_A) = \tilde{g}(x_B, x_\Gamma)$ is constant with respect to x_Γ , we see that $\tilde{f} = \Psi(\tilde{g})$ has a like property and belongs to $\mathfrak{L}_p(X_A)$. If δ has been taken sufficiently small, it is clear that $N_p(f - \tilde{f}) \leq \frac{1}{2}\epsilon$ by virtue of the continuity of Ψ . Now if $n \geq m$, m being the integer just determined in our choice of \tilde{f} , we see that $\tilde{h}_n(x_A) = L_{x_\Gamma}\tilde{f}(x_B, x_\Gamma) = \tilde{f}(x_B, x_\Gamma) = \tilde{f}(x_A)$ because $\tilde{f}(x_B, x_\Gamma)$ is constant with respect to x_Γ , $\Gamma = \{\alpha; \alpha > n\}$. Hence we have $N_p(f - h_n) = N_p(f - \tilde{f} + \tilde{h}_n - h_n) \leq N_p(f - \tilde{f}) + N_p(\tilde{h}_n - h_n)$ for $n \geq m$, by Minkowski's inequality. By our choice of \tilde{f} we have $N_p(f - \tilde{f}) \leq \frac{1}{2}\epsilon$ and $N_p(\tilde{h}_n - h_n) = (L_{x_A}|\tilde{h}_n(x_A) - h_n(x_A)|^p)^{1/p} = (L_{x_B}L_{x_\Gamma}|\tilde{f}(x_B, x_\Gamma) - f(x_B, x_\Gamma)|^p)^{1/p} = (L_{x_B}|L_{x_\Gamma}(\tilde{f}(x_B, x_\Gamma) - f(x_B, x_\Gamma))|^p)^{1/p} \leq (L_{x_B}L_{x_\Gamma}|\tilde{f}(x_B, x_\Gamma) - f(x_B, x_\Gamma)|^p)^{1/p} = (L_{x_A}|\tilde{f}(x_A) - f(x_A)|^p)^{1/p} = N_p(f - \tilde{f}) \leq \frac{1}{2}\epsilon$. Hence $N_p(f - h_n) \leq \epsilon$ for $n \geq m$, as we wished to show. A further result of Jessen will complete our discussion, namely:

- (10) (Jessen) the relations $\lim_{n \rightarrow \infty} g_n(x_A) = L_{x_A} f(x_A)$, $\lim_{n \rightarrow \infty} h_n(x_A) = f(x_A)$ hold almost everywhere in the pointwise sense.

We consider only h_n , modeling our treatment on that already given by Jessen⁶ for g_n . Since $\mathfrak{L}_p(X_A) \subset \mathfrak{L}(X_A)$ we may suppose that $p = 1$ in the present instance. The sequence $\{h_n\}$, being convergent to f in $\mathfrak{L}(X_A)$ has a subsequence which converges almost everywhere to f in the pointwise sense. Consequently $h = \limsup_{n \rightarrow \infty} h_n \geq f$ almost everywhere. By a

method which will be sketched below we show that at almost every point of the set $X^\lambda = \{x_A; h(x_A) > \lambda\}$ we must have $f(x_A) \geq \lambda$. It then follows that $\limsup_{n \rightarrow \infty} h_n(x_A) = f(x_A)$ almost everywhere. Replacing f by $-f$, we

have to replace h_n by $-h_n$. We therefore have $\liminf_{n \rightarrow \infty} h_n(x_A) = -\limsup_{n \rightarrow \infty} (-h_n(x_A)) = -(-f(x_A)) = f(x_A)$, a relation which completes the proof of the theorem. Reverting now to the detailed study of X^λ , we let $f_{np}^\lambda \in \mathfrak{L}(X_A)$ be the characteristic function of the set $\{x_A; h_{n+p}(x_A) > \lambda, h_k(x_A) \leq \lambda \text{ for } n \leq k \leq n+p-1\}$, $n = 1, 2, 3, \dots$ and $p = 0, 1, 2, \dots$. The characteristic function of X^λ is then expressible as $f^\lambda = \lim_{n \rightarrow \infty} \sum_{p=0}^{\infty} f_{np}^\lambda \in \mathfrak{L}(X_A)$. Since

h_k is constant with respect to x_l for $l \geq k+1$ we see that h_{n+p} and f_{np} are both constant with respect to x_l for $l \geq n+p+1$. Let g be an arbitrary function in $\mathfrak{L}(X_A)$ which is constant with respect to x_l for $l \geq m+1$ and which satisfies the inequalities $0 \leq g \leq 1$. Taking $n \geq m$ we note that $f_{np}^\lambda g$ is constant with respect to x_l for $l \geq n+p+1$ and hence can be multiplied into both members of the equation $h_{n+p}(x_A) = L_{x_A} f(x_B, x_A)$ to yield $h_{n+p}(x_A) f_{np}^\lambda(x_A) g(x_A) = L_{x_A} [f(x_B, x_A) f_{np}^\lambda(x_B, x_A) g(x_B, x_A)]$. Applying $L = L_{x_A}$ to both members of the latter equation we obtain $L(h_{n+p} f_{np}^\lambda g) = L(ff_{np}^\lambda g)$. Since $h_{n+p} > \lambda$ on the set where $f_{np}^\lambda = 1$ it follows that $\lambda L(f_{np}^\lambda g) \leq L(ff_{np}^\lambda g)$ and hence that $\lambda L(f^\lambda g) \leq L(ff^\lambda g)$. It is not difficult to determine a sequence of functions g of the kind admitted here which converges boundedly to the characteristic function $g^{\lambda-\epsilon}$ of the set $\{x_A; f(x_A) \leq \lambda - \epsilon\}$, exception being made as usual for points of a null set. Passage to the limit in the above inequality therefore yields $\lambda L(f^\lambda g^{\lambda-\epsilon}) \leq L(ff^\lambda g^{\lambda-\epsilon}) \leq (\lambda - \epsilon) L(f^\lambda g^{\lambda-\epsilon})$. Hence $\epsilon > 0$ implies $L(f^\lambda g^{\lambda-\epsilon}) = 0$; in other words the part of X^λ where $f(x_A) \leq \lambda - \epsilon$ is a null set. Thus we must have $f(x_A) \geq \lambda$ almost everywhere on X_A , as we claimed above.

¹ Stone, M. H., "Notes on Integration, I," these PROCEEDINGS, 34, 336-342 (1948); "Notes on Integration, II," *Ibid.* 447-455 (1948); cited here as I and II, respectively.

² The symbol x in these expressions denotes a bound variable.

³ Saks, S., *Theory of the Integral*, 2nd revised ed., Warszawa-Lwow, 1937, pp. 87-88.

⁴ Weil, A., *L'Intégration dans les Groupes Topologiques et ses Applications*, Paris, 1938, pp. 30-45, especially 42-45.

¹ While this result resembles one established by Ambrose, W., "Direct Sum Theorem for Haar Measures," *Trans. Am. Math. Soc.*, 61, 122-127 (1947), it is actually identical with the latter only in the case where Z is separable. The reason for the distinction which must be made in the non-separable case is indicated in the fourth footnote of II.

² Jessen, B., "The Theory of Integration in a Space of an Infinite Number of Dimensions," *Acta Mathematica*, 63, 249-323 (1934), especially 272-280.

³ $\mathfrak{E}(X_A)$ and E_A have in a general way the character of "projective limits" of the given $E(X_B)$ and E_B , the conditions (5) and (6) being "consistency conditions" essential to the constructive process.

ERRATUM

The eleventh (last) pentad of the second line of the value for $340!/10^{83}$ on p. 409 (August) of volume 34 of these PROCEEDINGS should read 85229 in place of 58229.

HORACE S. UHLER

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ENZYMATIC FIXATION OF CARBON DIOXIDE IN α -KETO-GLUTARIC ACID*

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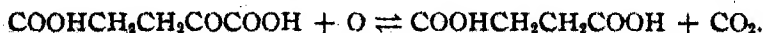
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During investigations on the heterotrophic assimilation of CO_2 by *Escherichia coli* and *Aerobacter aerogenes*, it was shown that the C_5 compounds, α -ketoglutaric acid and its precursor, glutamic acid, were more effective than the other members of the Krebs cycle or their precursors in replacing CO_2 in the metabolic requirements of the organisms.¹ It was proposed that a fixation over and above the Wood and Werkman reaction takes place and that this reaction may be of great importance to the cell. The present purpose is to report the enzymatic exchange of C^{13}O_2 with the carboxyl group of α -ketoglutaric acid and to show a new type of heterotrophic assimilation of CO_2 involving a C_4 and C_1 addition.

A cell-free enzyme preparation from *E. coli* decarboxylates α -ketoglutarate to succinate and CO_2 in the presence of malonic acid as an inhibitor of succinate oxidation. Under proper conditions the same preparation fixes C^{13}O_2 in the carboxyl group adjacent to the carbonyl carbon of the C_5 acid.

Any exchange reaction involving CO_2 and resulting in the formation of a carbon-to-carbon linkage is considered to be a fixation reaction. Attempts to demonstrate the carboxylation of succinate have thus far failed, probably because the equilibrium of the reaction is far to the side of decarboxylation and the quantities of α -ketoglutarate formed are too small to detect. A phosphorylated form of succinate is apparently involved since adenosine triphosphate enhances the fixation. The reaction as studied in the presence of malonate is a reversible oxidative decarboxylation involving a half mole of oxygen uptake for each mole of α -ketoglutaric acid utilized for the production of a mole of succinate and a mole of CO_2 . Thus,



Methods.—*E. coli* was grown for 16–18 hrs. at 30°C . in a medium con-

taining 0.8% glucose, 0.4% $(\text{NH}_4)_2\text{SO}_4$, 0.8% KH_2PO_4 , 0.2% yeast extract and 10% tap water at an initial pH of 7.0. The cells were then harvested and ground with powdered glass and subsequently extracted with phosphate buffer solution (Kalmitsky, *et al.*²).

The exchange reactions were carried out in 125 ml. Warburg-Barcroft reaction vessels with two side arms. The final concentration of the α -ketoglutarate was 0.01 *M* and that of malonate 0.05 *M*. Sodium salts of both acids were used. Depending on the activity of the juice, a quantity varying between 5 and 10 ml. was added to each flask. The mixture was buffered with 0.2 *M* phosphate at pH 6.6 and 0.07 *M* $\text{NaHC}^{18}\text{O}_3$. Appropriate concentrations of the two buffers were mixed with the substrate and enzyme after temperature equilibration (30.4°C.) had been established. pH of the resulting mixture was approximately 7.2 and the volume 25 or 30 ml.

The reaction was allowed to continue until approximately half of the substrate was utilized. The residual CO_2 in the mixture was then determined by aeration. Addition of the necessary reagents was made during the course of aeration and heating. The C^{18}O_2 was liberated by the addition of 6 *N* H_2SO_4 and collected in 8 ml. of 4 *N* carbonate-free NaOH in specially constructed carbon dioxide absorption tubes.

Since both malonate and α -ketoglutarate are decarboxylated by the same reagents, the former was removed before decarboxylating the keto acid by the addition of 10 ml. of 0.025 *M* sodium bisulfite to the reaction mixture from which the residual CO_2 had been removed. The deproteinized sample was extracted for 72 hrs. with ether. The ether extract contained all of the original malonate as subsequently determined, plus the other dicarboxylic acid (succinic) formed during the reaction.

To the extracted solution was added 0.75 mM. of $\text{NaHC}^{12}\text{O}_3$, further acidified with H_2SO_4 , heated and aerated for 15 minutes. The use of C^{12}O_2 rinse precluded residual C^{18}O_2 remaining to interfere with the isotope determination of the carboxyl carbon. The C^{18}O_2 content of the rinse was always determined.

α -Ketoglutaric acid was degraded with ceric sulfate and KMnO_4 to succinate and CO_2 . The CO_2 originates from the carboxyl adjacent to the carbonyl group; it was collected and the C^{13} content determined on the mass spectrometer.

Experimental.—Succinic acid and carbon dioxide are the products formed by the aerobic dissimilation of α -ketoglutaric acid in the presence of malonate by the cell-free enzyme preparation of *E. coli* (table 1). The reaction is similar to that reported by Ochoa³ using cat heart as the source of α -ketoglutaric dehydrogenase. When the action of the bacterial succinic dehydrogenase is blocked by sodium malonate, one molecule of succinate and one molecule of CO_2 are formed for each atom of oxygen taken

up in the oxidative decarboxylation of the keto acid. Succinate determinations by the silver salt method or by the oxidation with succinic dehydrogenase obtained from beef heart agreed well with the values for oxygen uptake and CO_2 evolved. No attempts were made to determine the products when malonate was omitted since the respiratory quotient of 1.2 to 1.3 obtained (theoretical 1.25) indicated a complete oxidation of α -ketoglutarate to CO_2 and water.

TABLE 1
OXIDATIVE DECARBOXYLATION OF α -KETOGLUTARATE BY A CELL-FREE ENZYME
PREPARATION OF *E. coli*

MATERIAL DETERMINED	WITH MALONATE*	R.Q.		WITHOUT MALONATE†	R.Q.	
		THEORY	EXPTL.		THEORY	EXPTL.
α -Ketoglutarate utilized; mM.	0.02	0.002
Succinic acid formed; mM.	0.017	0.0
O_2 uptake; μl .	109	2.0	2.1	103	1.25	1.30
CO_2 liberated; μl .	239			135		

Total volume of reactants 2.8 ml. 0.0035 *M* α -ketoglutarate, 0.05 *M* PO_4 buffer, pH 6.8, 1 ml. bacterial juice per cup. Temp., 30.4°C.

All values are corrected for enzyme blanks.

* 0.031 *M* sodium malonate.

† Time, 2 hrs.

The cell-free enzyme preparations are generally not highly active on α -ketoglutarate or succinate but were used because malonic acid does not block the oxidation of succinate when the intact bacterial cell is employed (table 2). It was assumed that malonate did not penetrate the cell, therefore the bacteria were treated with various solvents to increase the permeability of the cell wall to the inhibitor. However, no satisfactory results were obtained. Quastel and Wooldridge⁴ have shown that exposure of *E. coli* to toluene brings about a number of inactivations. The enzymes for lactic, succinic and formic acids were found intact. Our results are in agreement. Toluene and acetone treated cells remain active on succinate and become permeable to malonate; however, the same cells show no activity on α -ketoglutarate. *n*-Propyl alcohol destroys both enzymes.

Recently, Klotz and Tietze⁵ reported on the inhibition of succinic acid oxidation by structurally related sulfonic acids using rat liver homogenates. No such inhibition was noted with bacterial cells (table 3). However, in the case of bacterial juices, high concentrations are effective. The endogenous activity of the juice remains essentially unchanged even when a concentration as 0.08 *M* of inhibitor is used. Malonate not only inhibits succinic dehydrogenase but reduces the endogenous activity as well. Since toluene-treated cells remain active on succinic acid, this treatment does

TABLE 2
EFFECT OF MALONIC ACID ON SUCCINIC DEHYDROGENASE

ENZYME PREPARATION	METHOD OF INVESTIGATION					THUNBERG; REDUCTION		
	MANOMETRIC; OXYGEN UPTAKE, μ L.					OF M.B.		
	SUBSTRATES					% REDUCTION		
	SUCCI- NATE	SUCCI- NATE + MALO- NATE*	α -KETO- GLUTARATE	α -KETO- GLUTARATE + MAL- ONATE	MALO- NATE	TIME, HRS.	SUCCI- NATE	SUCCI- NATE + MALO- NATE
Suspension of washed <i>E. coli</i>	446	516	186	193	30	2	90	90
Toluene-treated sus- pension of <i>E. coli</i>	103	3	18	-10	0	2	80	0
Acetone-treated sus- pension of <i>E. coli</i>	201	63	62	33
<i>n</i> -Propyl alcohol- treated suspension of <i>E. coli</i>	-6	-2	23	6	0
Cell-free enzyme preparation of <i>E. coli</i>	93	65	257	153	-6	0.4	90	0

Manometric data: Total volume of reactants 2.3 ml. 0.0043 *M* α -ketoglutarate and succinate, respectively, 0.05 *M* PO_4 buffer, pH 7.0. Bacterial suspensions were added at the rate of 30 mg. per cup, dry weight, bacterial juice, 1 ml. per cup. Temp., 30.4°C.

Thunberg data: Total volume 6.0 ml. 0.0033 *M* succinate, 0.0066 *M* malonate, 0.066 *M* PO_4 buffer, pH 7.2. Bacterial suspensions, 0.5 ml. 10% suspension wet weight. Dilution of methylene blue 1/5000.

* 0.0086 *M* sodium malonate.

TABLE 3
OXIDATION OF SUCCINIC ACID IN THE PRESENCE OF 1,2-ETHANE DISULFONIC ACID

ENZYME PREPARATION	SUBSTRATE	OXYGEN UPTAKE, μ L.	METHYLENE BLUE TIME, HRS.	% REDUCTION
Suspension of washed <i>E. coli</i>	Succinate	312	0.6	75
			0.4	95
	Succinate + inhibitor	352	0.3	95
Toluene-treated <i>E. coli</i>	Succinate	107	2	75
	Succinate + inhibitor	103	2	80
Cell-free enzyme preparation of <i>E. coli</i>	Succinate	224
	Succinate + inhibitor	60

Manometric data: Total volume 2.3 ml. 0.0043 *M* succinate, 0.05 *M* PO_4 buffer, pH 7.2, 0.08 *M* 1,2-ethane disulfonic acid. Bacterial suspensions were added at the rate of 30 mg. per cup dry weight, bacterial juice—1 cc. per cup. Temp., 30.4°C.

Thunberg data: Total volume 6.0 ml. 0.0033 *M* succinate, 0.16 *M* inhibitor, 0.066 *M* PO_4 buffer, pH 7.0. Bacterial suspensions, 0.5 cc. of a 10% suspension wet weight. Dilution of methylene blue 1/5000.

not alter permeability to 1,2-ethanedithionyl acid. Type of the inhibition is yet to be determined. Apparently the sulfonate ion forms an enzyme complex of almost the same strength as that with malonate.

Because of the difficulties with intact cells, it was necessary to employ a cell-free preparation which not only oxidatively decarboxylates α -ketoglutarate to succinate and CO_2 but under the proper experimental conditions carboxylates succinate to form the C_5 keto acid (table 4).

TABLE 4
EXCHANGE OF HEAVY CARBON DIOXIDE IN THE CARBOXYL GROUP OF α -KETOGLOUTARIC ACID DURING ENZYMIC DECARBOXYLATION

EXPT. NO.	ATP ADDED, mg.	α -KETOGLOUTARATE ADDED, mM.	α -COOH OF α -KETOGLOUTARATE			RESIDUAL $\text{NaHC}^{13}\text{O}_3$			MALONATE		COOH GROUPS
			RECOVERED, mM.	EXCESS C^{13} , %	C^{13} FIXED, mM. $\times 10^4$	EXCESS C^{13} , %	RECOVERED,* mM.	EXCESS C^{13} , %	ADDED, mM.	RECOVERED, mM.	
1	..	0.25	0.16	0.171	2.7	2.79	0.75	0.00	1.25	1.24	0.00
2	..	0.25	0.15	0.00	0.0	1.25
3	..	0.25	0.09	0.06	0.5	Not added
4a†	..	0.25	0.18	0.102	1.8	1.25	1.23	..
4b	40	0.25	0.19	0.372	7.0	1.25	1.25	..

Reactions were carried on in 125-ml. Erlenmeyer flasks with two side arms which were attached to the Warburg-Barcroft manometers. The final concentration of the α -ketoglutarate was 0.01 *M* and that of malonate 0.05 *M*. Depending on the activity of the juice, a quantity varying between 5 and 10 ml. was added to each flask. The mixture was buffered with 0.2 *M* phosphate, pH 6.6 and 0.07 *M* $\text{NaHC}^{13}\text{O}_3$ (3.113% excess). Appropriate concentrations of the two buffers were mixed with the substrate and enzyme after temperature equilibrium (30.4°C.) had been reached. The pH of the resulting mixture was approximately 7.2 and the volume 25 or 30 ml.

* mM. represent amounts recovered after treatment with $\text{NaHC}^{13}\text{O}_3$.

† Experiments 4a and 4b were carried out simultaneously.

It is apparent that the decarboxylation of α -ketoglutaric acid is reversible. The per cent excess of C^{13} in the α -carboxyl of the keto acid was much higher when the reaction was conducted in the presence of malonate and adenosine triphosphate. In the absence of malonate the products of the reaction were water and CO_2 . As soon as succinate was formed, it was oxidized. No detectable carboxylation of succinate occurred, consequently the reaction is not recognized as reversible. Since the error of the mass spectrometer is ≈ 0.02 , a per cent excess of 0.06% C^{13} is questionable.

In the presence of malonate succinic acid accumulates to enhance the fixation of CO_2 . At approximately half time of the reaction carboxylation is readily detectable.

In common with other carboxylations, this one is endergonic as is borne out by its enhancement by adenosine triphosphate (table 4).

It should be pointed out that at times reversibility could not be demonstrated. This may have been due to faulty procedure in the preparation of the juice. For example, when the juice is not centrifuged long enough to remove all of the unground cells, enough of them will be present to oxidize much of the succinate formed since their succinic dehydrogenase is not blocked by malonate, and consequently no CO_2 can be shown to be fixed. Partial denaturation of the enzyme or some component of the enzyme system may also account for experiment 2 in table 4. However, the discrepancies in this experiment are not too serious when compared with the overwhelming evidence for the reversibility (Expts. 1, 3, 4a and 4b). Any significant failure of malonate to block succinate dehydrogenation may result in fixation of carbon dioxide in the α -ketoglutaric acid. Our previous experience would lead us to believe that this has not occurred under the conditions.

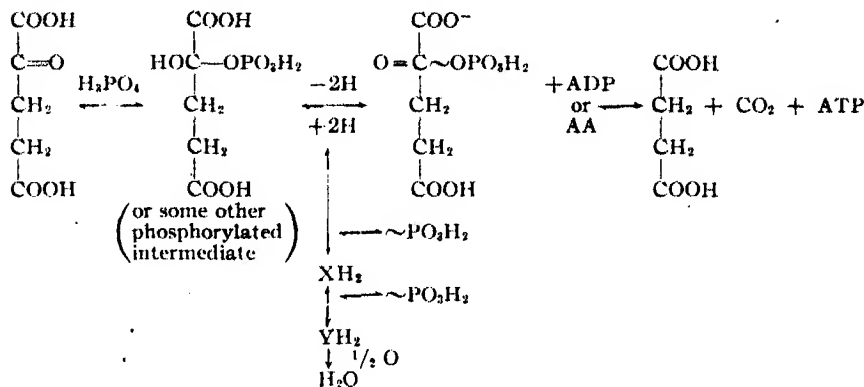
Discussion.—Although the present status of the Krebs cycle in bacterial metabolism is still uncertain, the evidence thus far obtained favors the occurrence of such a cycle in certain bacteria at least in principle. The results of Ajl, *et al.*,¹ on the replacement of CO_2 are of particular significance in this connection since metabolites normally occurring in that cycle are able to replace CO_2 thus showing not only a common function of these compounds in replacing CO_2 , but potential interconversions among these compounds as well.

Every reaction of the Krebs cycle except the one studied has been shown to be reversible. According to the present findings, a complete reversal of the oxidative degradation of foodstuff would now be possible. By carboxylation and reduction, α -ketoglutaric acid would be converted to citric acid and the latter would split into acetic and oxalacetic acids as shown by Brewer and Werkman.⁶ Further, oxalacetic acid would be reduced to succinic acid by way of malic and fumaric acids, and the succinate thus formed would be converted to α -ketoglutaric acid by reductive carboxylation. In this way, α -ketoglutaric acid would be constantly regenerated. Therefore, CO_2 and H_2 entering the cycle at various points during metabolism would emerge as pyruvate which could then be used for the synthesis of carbohydrates.

In a system devoid of CO_2 , α -ketoglutaric acid may function in one or two ways. First, it may be oxidatively decarboxylated to yield succinic acid and CO_2 , the former being utilized by the organism in place of CO_2 , and secondly, the keto acid may function as a substrate for transamination, a mechanism responsible for the interconversion of proteins and carbohydrates. Since growth depends on the presence of carbon dioxide, which is replaced by α -ketoglutarate to a greater extent than any of the C₄ acids,

it is entirely possible that during the normal metabolic processes of the bacterial cell the C_1 to C_4 addition may be of greater importance to the cell than some of the other fixation reactions known to occur.

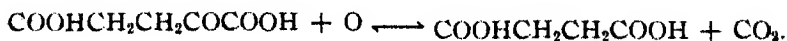
The mechanism of the reversibility may be represented:



where X and Y are intermediary hydrogen acceptors.

Of the 50 energy-rich phosphate bonds that are generated when one molecule of glucose is completely oxidized, not more than two are formed during the initial anaerobic stages that lead to the formation of lactic acid. The residual 48 bonds therefore must arise during the subsequent oxidative stages. Twenty-four (\sim) must be generated from each molecule of lactate. Lactate is dehydrogenated to pyruvate and the keto acid oxidatively degraded through the Krebs cycle. But of all the reactions involved in this cycle only two are known to undergo oxidative decarboxylations yielding energy-rich phosphate bonds, i.e., the oxidative decarboxylation of pyruvate and of α -ketoglutarate. It follows that some 22 additional energy-rich phosphate bonds are formed as a result of other reactions. It is postulated that the additional bonds are formed during the transfer of electrons from the primary acceptor to oxygen. If such bonds are generated, their energy could conceivably be used to reverse the intermediary steps of the reaction studied. That this may actually be the case is borne out by the fact that ATP enhances the reversibility (table 4) by increasing the concentration of energy-rich phosphate bonds.

Summary.—Reversibility of the following reaction has been demonstrated with a cell-free enzyme preparation of *E. coli*.



Adenosine triphosphate enhances the reversibility.

Occurrence of this reaction explains the function of α -ketoglutaric acid in replacing CO_2 .

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¹ Aji, S. J., White, A. G. C., and Werkman, C. H., *J. Bact.*, **54**, 23 (1947).

² Kalnitsky, G., Utter, M. F., and Werkman, C. H., *Ibid.*, **49**, 595-602 (1947).

³ Ochoa, S., *J. Biol. Chem.*, **155**, 87-100 (1944).

⁴ Quastel, J. H., and Wooldridge, W. R., *Biochem. J.*, **22**, 689-702 (1922).

⁵ Klotz, I. M., and Tietze, F., *J. Biol. Chem.*, **168**, 399-400 (1947).

⁶ Brewer, C. R., and Werkman, C. H., *Enzymologia*, **6**, 273-281 (1939).

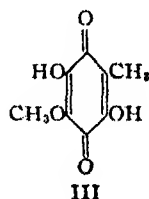
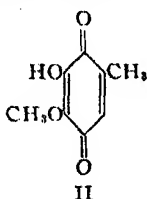
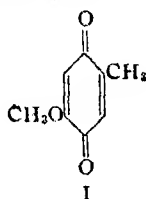
ANTIBIOTIC SUBSTANCES FROM BASIDIOMYCETES III. *COPRINUS SIMILIS* AND *LENTINUS DEGENER**

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In the course of our investigations of the production of antibiotic substances by Basidiomycetes, two fungi, *Coprinus similis* and *Lentinus degener*,¹ were found to form the same antibacterial substance. This has been determined to be 5-methoxy- β -toluquinone (I).



The identification of this compound as a mold product is of particular interest because two related compounds, fumigatin (II) and spinulosin (III), have previously been obtained from the culture liquids of fungi. Fumigatin is produced by *Aspergillus fumigatus*² spinulosin by *Penicillium spinulosin*³ and by *A. fumigatus*.⁴

Antibacterial Activity of Culture Liquids.—Each of the two fungi was grown at 25°C. in 2800-ml. Fernbach flasks containing 1 liter of modified Czapek-Doz medium with dextrose and corn steep solids on coils of beechwood shavings⁵ which furnished mechanical support for the mycelium.

Cultivated under these conditions, *C. similis* produced in about one month culture liquids with an activity of 128 or 256 dilution units per milliliter when tested against *Staphylococcus aureus* by serial dilution. The antibacterial activity remained essentially unchanged in such cultures for two months or more. When the activity of the culture liquids reached

128 or 256 dilution units, the old culture liquid was decanted and replaced with fresh sterile corn steep medium. Within seven to ten days, sufficient antibacterial activity had developed to justify further reflooding. Mats sixty-one days old were successfully reflooded and some mats were reflooded three successive times. The maximum activity observed for our cultures was 256 dilution units per milliliter.

Culture liquids of *L. degener* developed an activity 2 to 4 times that observed for those of *C. similis*. In three to four weeks, the antibacterial activity of the culture liquids of *L. degener* was, as a rule, 256 dilution units per milliliter which with further incubation of a week or two reached 500 or 1000 dilution units and, on occasion, even 2000 dilution units per milliliter. If mats which produced culture liquids of activity such as mentioned were reflooded with fresh sterile medium, the new culture liquid evidenced an activity of 256 to 1000 dilution units per milliliter in five to ten days. Some mats were successfully reflooded 3 successive times.

Although both fungi produced culture liquids with substantial antibacterial potency, their activity as determined by the streak and disc methods was unsatisfactory. In fact, if the results from the streak and disc methods had been used as a standard of judgment, neither fungus might have been considered worthy of further investigation.⁶

Preliminary tests with the culture liquids of *C. similis* indicated that the antibacterial substance was largely destroyed by boiling at pH 8.8 or 11.0, and was destroyed in part by boiling at pH 4.1. These facts were borne in mind in efforts to isolate the material.

Isolation of Crystalline Material.—It was found that the antibacterial material could be removed almost quantitatively by extracting the culture liquid with ethyl ether or chloroform. From the ether or chloroform extracts a golden-yellow crystalline material was obtained either as plates or clusters of short prisms. These crystals had a characteristic aromatic odor which could be detected from the culture liquids also.

A procedure developed for isolating the crystalline material consisted of adjusting the pH of the culture liquid which was originally between 3.5 and 5.0 to 5.5 or 6.0, and extracting three times with one-fifth the volume of chloroform each time. The chloroform was removed under reduced pressure, and the crystalline residue was recrystallized from 95 per cent alcohol with addition of charcoal. The final product was dried *in vacuo* and stored in evacuated vials in the refrigerator to avoid possible oxidation.

In the following table, the results of the extraction of the culture fluid from each fungus is given:

FUNGUS	AV. SIZE BATCH, LITERS	NO. OF BATCHES	AV. ACTIVITY, DILUTION UNITS PER ML. OF ORIGINAL CULTURE FLUID			ISOLATED, G. PER LITER CULTURE FLUID
			CULTURE LIQUID	CRUDE CRYSTALS	PURIFIED CRYSTALS	
<i>C. similis</i>	8.5	2	128	192	303	0.12
<i>L. degener</i>	15.0	11	1005	1150	1060	0.37

For example, two batches of culture liquid from *C. similis* were worked up. They averaged 8.5 liters per batch and the culture liquid had an average activity of 128 dilution units per milliliter. The total antibacterial activity of the crude crystals was equivalent to 192 dilution units per milliliter of the original culture liquid, indicating complete or nearly complete extraction. The total activity of the purified crystals was equivalent to 303 dilution units per milliliter of culture liquid, which is within the probable error of the serial dilution method used in determining activity. The purified crystals recovered amounted to 0.12 g. per liter of culture fluid.

Identity of Crystals from Two Fungi.—The identity of the yellow crystalline material from the culture liquid of *L. degener* and that from the culture liquid of *C. similis* was established by mixed melting points of purified samples of crystals obtained from the two sources, by carbon and hydrogen analyses of both samples and by a comparison of the bacterial spectra of crystalline material obtained from culture liquids of each fungus. No evidence for the production of more than one antibacterial substance by either fungus was observed.

Bacterial Spectrum.—The inhibitory concentration at twenty-four hours in micrograms per milliliter as found in three determinations for crystals obtained from the culture fluid of *C. similis* and from that of *L. degener*, was as follows. Tests of the same number were run simultaneously. The crystals were dissolved in 10, 15 or 20 per cent alcohol, and the determinations were made by serial dilution.

ORGANISM	MINIMUM INHIBITORY CONCENTRATIONS IN μ G. PER ML. CRYSTALS FROM <i>C. similis</i>			MINIMUM INHIBITORY CONCENTRATIONS IN μ G. PER ML. CRYSTALS FROM <i>L. degener</i>		
	(1)	(2)	(3)	(1)	(2)	(3)
<i>Bacillus mycoides</i>	32	...	16	64	...	16
<i>Bacillus subtilis</i>	2	2	4	4	2	4
<i>Escherichia coli</i>	64	64	64	128	64	32
<i>Klebsiella pneumoniae</i>	16	64	32	64	64	16
<i>Pseudomonas aeruginosa</i>	256	128	256	256	128	256
<i>Mycobacterium smegma</i>	128	64	128	128	128	64
<i>Staph. aureus</i>	1.0	0.5	1.0	0.5	0.5	0.5

Antifungal Action.—The effect of crystals prepared from the culture liquids of *C. similis* and *L. degener* on the inhibition of several fungi was determined. A spore suspension of each fungus was tested by serial dilution in 1-ml. quantities of a peptone medium⁷ at pH 6.0. *Trichophyton mentagrophytes* was incubated at 30°C.; the other fungi at 25°C. The activity of both samples of crystals was essentially the same. *T. mentagrophytes* was the most sensitive; *Gliomastix convoluta*, *Stemphylium consortiale* and *Penicillium notatum*, the most resistant.

	MINIMUM INHIBITORY CONCENTRATION ^a IN μ O. PER ML.		
	24 HRS.	AT END OF 42 HRS.	72 HRS.
<i>Staph. aureus</i>	1 (1)	1 (1)
<i>A. niger</i>	16	32	32
<i>Chaetomium globosum</i> (USDA 1042.4)	16 (16)	16 (32)
<i>Gl. convoluta</i> (PQMD 4c)	64 (64)	128 (128)	128 (128)
<i>Memnoniella echinata</i> (PQMD 1c)	32 (32)	32 (64)
<i>Myrothecium verrucaria</i> (USDA 1334.2)	64 (64)	64 (64)
<i>St. consortiale</i> (PQMD 41b)	64 (128)	128 (128)
<i>P. notatum</i>	128 (128)	128 (128)	128 (128)
<i>Phycomyces blakesleeana</i> (+)	32	32	64
<i>T. mentagrophytes</i>	4 (4)	8 (8)

Identification of Crystalline Material.—The identification of the crystalline material as 5-methoxy-*p*-toluquinone was based on the following evidence.

1. The substance showed typical quinone behavior: its solution in concentrated sulfuric acid was bright orange-red; an alkaline solution darkened rapidly to a purplish brown color and the compound could not be recovered on acidification; it reacted with carbonyl reagents; it took up bromine in carbon tetrachloride; it yielded a purple crystalline derivative with aniline; it gave a blue-violet color changing to blue-green and finally reddish-brown on treatment with ethyl cyanoacetate and ammoniacal ethanol.⁸

2. The melting point was in agreement. Luff, Perkin and Robinson¹⁰ report a melting point of 170–172°C. with previous softening at 165° for (I).

3. Analyses established an empirical formula of $C_8H_8O_3$.

4. The compound underwent the same reactions as those reported for (I) and gave products with the reported melting points

(a) On reduction with sulfur dioxide, a white solid was obtained which crystallized from water in long, fine needles of melting point 122–124°C. The melting point reported for the reduction product of (I) is 124°C.¹⁰

(b) "Thiele acetylation" yielded a white solid which crystallized in prismatic needles of melting point 91–92°C. Erdtman¹¹ reports 91–92°C. as the melting point for 4-methoxy-2,3,5-triacetoxytoluene obtained by this method from (I).

(c) Treatment with acetylchloride and acetic acid gave a white solid which crystallized from acetic acid in fine needles of melting point 126–127°C. The melting point of 3-chloro-2,5-diacetoxy-4-methoxytoluene obtained from (I) by this method is 126°C.¹¹

5. The positions and heights of the maxima in the absorption spectrum are in agreement with those reported for (I).¹²

Oxford¹³ compared the antibacterial activity of a number of derivatives of toluquinone and benzoquinone. He found 5-methoxy-*p*-toluquinone¹⁴ to be one of the more active compounds effective on gram-positive organ-

isms but without striking action on gram-negative bacteria. It was more active than fumigatin which in turn had a greater antibacterial effect than spinulosin. The inhibitory concentrations reported by Oxford for *Staph. aureus* are one-third of those we found for this compound or less. This is probably because of differences in strains of bacteria and differences in methods of test.

Some of its characteristics militate against the possible therapeutic application of 5-methoxy-*p*-toluquinone. Its solubility in water or saline was low (0.5 mg. per milliliter). When exposed to 5 per cent blood for three hours at 37°C., its activity was reduced on the average by 50 per cent. A few preliminary tests indicated that 25 mg. per kilogram killed one-half the mice injected intravenously within five days.

* This investigation was supported in part by grants from the Albert H. and Jessie D. Wiggin Foundation and from The Commonwealth Fund.

¹ We are indebted to Dr. José Emilio Santos Pinto Lopes, Instituto Botanico, Lisbon, Portugal, for the culture of *C. similis*, and to Professor Roger Heim, Museum National d'Histoire Naturelle, Paris, for that of *L. degener*.

² Anslow, W. K., and Raistrick, H., *Biochem. J.*, **32**, 687-696 (1938).

³ Birkinshaw, J. H., and Raistrick, H., *Roy. Soc. Lon. Trans.*, **B220**, 245-254 (1931).

⁴ Anslow, W. K., and Raistrick, H., *Biochem. J.*, **32**, 2288-2289 (1938).

⁵ Robbins, W. J., Kavanagh, F., and Hervey, A., these PROCEEDINGS, **33**, 171-176 (1947).

⁶ Hervey, A. H., *Bull. Torrey Bot. Club*, **74**, 476-503 (1947).

⁷ This medium contained per liter 1.5 g. KH_2PO_4 , 0.5 g. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 50 g. dextrose, 2 g. neopeptone, 1500 m μ moles of thiamine, and a mixture of minor mineral elements as used in this laboratory.

⁸ The figures in parentheses are the values for crystals from *L. degener*; the others are those for crystals from *C. similis*. We are indebted to Dr. W. L. White for cultures of the fungi with USDA or PQMD numbers.

⁹ Craven, R., *J. Chem. Soc.*, **1931**, 1605-1606 (1931).

¹⁰ Luff, B. D., Perkin, W. H., Jr., and Robinson, R., *Ibid.*, **97**, 1131-1140 (1910).

¹¹ Erdtman, H. G. H., *Roy. Soc. Lon. Proc.*, **A143**, 177-191 (1933).

¹² Braude, E. A., *J. Chem. Soc.*, **1945**, 490-497 (1945).

¹³ Oxford, A. E., *Chem. and Ind.*, **61**, 189-192 (1942).

¹⁴ According to the system of nomenclature used by Oxford, this compound is 4-methoxy-*p*-toluquinone.

THE BEARING OF THE LIVING METASEQUOIA ON PROBLEMS
OF TERTIARY PALEOBOTANY

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The discovery of huge living trees in California in 1769, followed by the naming and description of the coast redwood, *Sequoia sempervirens*, by Endlicher in 1847,¹ served as a prelude to the recognition of fossil redwoods in many parts of the northern hemisphere. Leafy shoots from the Oligocene of France, originally assigned to *Taxites langsdorffii* by Brongniart² were correctly transferred to the genus *Sequoia* by Heer in 1855.³ Heer also identified as members of this genus specimens sent to him from the collections of polar explorers, from Tertiary deposits in Greenland, Iceland, Spitzbergen, Siberia, Sachalin, northern Canada and Grinnell Land.⁴ Bringing to North America much of the tradition of European paleobotany, Lesquereux recognized several species of fossil *Sequoia* in the western United States, including the widely distributed *S. langsdorffii*.⁵ During ensuing years the occurrence of *Sequoia* has been widely noted over the northern hemisphere, at middle latitudes in rocks assigned to middle Tertiary, and at high latitudes in rocks of older Tertiary and Cretaceous age. From the pattern of its occurrence during these later periods of earth history, the paths of its migration southward have been charted.^{6, 7} Its environment in past ages has been reconstructed from comparisons with the modern habitat of the coast redwood in California and Oregon.

A fossil cone described as *S. heerii* by Lesquereux⁸ from beds of Oligocene age on Medicine Lodge Creek (Sage Creek), Montana, differs from other American cones referred to *Sequoia* in its attachment on a "naked pedicel." Lesquereux believed that the absence of needles on this "pedicel" (if it had had needles it could not have been properly so designated) had resulted from maceration. In later years identical stalked cones have been found at other Tertiary localities in western North America, such as Bridge Creek, Oregon, and Elko, Nevada; some of the cones figured by Heer as *S. nordenskioldi* from the Eocene of Spitzbergen,⁹ and as *S. langsdorffii*¹⁰ and *S. brevifolia*¹¹ from the Eocene of Greenland are also borne on stems lacking needles. For some time it has been apparent that this type of *Sequoia* cone is readily distinguishable from those of the living species and of other American fossil species; one of my students has even suggested that there are differences sufficient to justify establishing a new genus.¹²

In 1936 Endo¹³ pointed out for the first time a significant character in a

cone of this type, assigned to *S. japonica* from later Tertiary deposits of Japan and Korea. His description and remarks are as follows:

"Description: Cone rather small, spherical, ca. 16 mm. in diameter; cone-scales ca. 16 in number, arranged in 4 longitudinal rows, each row with 4 scales; escutcheon hexagonal, transversely grooved and radially wrinkled, 10 mm. wide, 3 mm. high. Peduncle stout, 2 mm. in diameter.

"Leaves with decurrent base, sessile, arranged in 2 lines; linear, bluntly mucronate at apex, 10 mm. long, 2 mm. wide; upper surface slightly furrowed along the midrib.

"Remarks: The arrangement of the cone-scales in the present species is in longitudinal rows, while being usually spiral in most other species. It has this characteristic feature in common with the cones from the Miocene of John Day valley, once described by Lesquereux as belonging to *Sequoia langsdorffii*; unfortunately the latter is too imperfect for farther comparison with the present materials."

Endo's use of the term "peduncle" is unfortunate, since the stems to which the cones are attached appear in at least two specimens (figures 6, 13) to bear leaves. They may best be considered leafy shoots bearing terminal cones. An obscure photograph (figure 12) of a vegetative shoot shows its needles in opposite position, although Endo does not mention this feature in his description of the leaves. In fact, many fossil shoots with oppositely placed needles had been figured as far back as the days of Heer with no reference to this readily observable character.

It remained for Miki¹⁴ to found a new genus, *Metasequoia*, in 1941 on the basis of stalked cones and leafy shoots from Pliocene deposits at Osusawa and elsewhere in the clay beds of Central Hondo. His description and discussion (in altered sequence) are here quoted:

"Character: Cone pedunculate, scale decussate, shield-form; peduncle with distichous scars of leaves and scaly leaves at the base. Shoot deciduous; leaf distichous, linear, obtuse, petiolate; stomata parallel to the midrib.

"The remains have usually been referred to *Sequoia* or *Taxodium*, indeed the cone is like that of *Sequoia* and the foliage shoot is somewhat like those of *Taxodium*.

"The cones were never found connected to branches, but as the leaf-scars on the peduncle are also distichous, it is conceivable that the cones and the shoots belonged to the same plant. The foliated shoots seem to be lateral branches shedding in autumn, because their length is usually constant and the proximal end is covered by scaly leaves, although they have no scaly bud on the top and the branches two or more years old have two or more bud scars on the nearly same point as in Fig. 8 Ge.

"The cone is distinguished from that of *Sequoia* by the decussate arrangement of scales and by the delicate peduncle having scale leaves at the base. The foliated shoot differs from *Sequoia* by distichous arrangement of leaves and by the brittle petiole. At a glance the shape of the shoot of fragmental remain seems to be *Taxodium* or *Cephalotaxus* but it differs from *Taxodium* by distichous leaf and parallel arrangement of stomata on it and from *Cephalotaxus* by short delicate shoot without scaly bud at the terminal and by the obtuse top of leaf.

"The decussate arrangement of cone-scales is not found in living *Taxodiaceae*, but a common character in *Cupressaceae*. The shedding of lateral foliated shoot with linear leaves is common in *Glyptostrobus* and *Taxodium*. So it is sure that the remains

belong to Taxodiaceae but as the characters do not harmonize with those of the living forms, a new genus *Metasequoia* is established."

Since several of the cone-stems which he figures (*A—g, h, i, j, k*) show scaly leaves or leaf scars, it seems clear that they are modified leafy shoots rather than peduncles. Miki uses the term "distichous" to describe the position of needles on the foliage shoots; whether he meant to indicate their opposite position (and I am inclined to believe from his phrasing that he did) is uncertain, but in any event the term carries no such implication; Sargent¹⁵ even defines "distichous" as "leaves arranged alternately in two vertical ranks," though the word may be equally well used for opposite position. The significant feature of Miki's description is his recognition of the association of cones bearing decussate scales with leafy shoots which were deciduous. He assigns two species to *Metasequoia*, of which one, *M. disticha*, was originally described as *S. disticha* by Heer¹⁶ from the Eocene of Spitzbergen on the basis of leafy shoots. The second, *M. japonica*, is the species originally described by Endo as *S. japonica* from Miocene and Pliocene deposits in Japan and Korea; it is distinguished from *M. disticha* by the shape and fewer numbers of its cone-scales, distinctions which may not prove to be of specific significance in the light of our present knowledge of the genus. Miki's assignment of these fossil cones and shoots to a new genus, and his conclusion* that even though they do not occur in direct attachment they are parts of the same deciduous plant, represent an outstanding discovery in Tertiary paleobotany. It is all the more remarkable, coming as it did only a few years before a second major discovery.

Miki's paper had probably not been read by any paleobotanist or botanist outside of Asia when it was announced, in the spring of 1946, that three trees of *Metasequoia* had been found living in Central China. Tsang Wang, attached to the Bureau of Forestry of the Ministry of Agriculture, had brought back to Nanking specimens collected at the village of Mo-tao-chi, in eastern Szechuan, about 140 miles northeast of Chungking. This material was studied by W. C. Cheng, Professor of Forestry at National Central University, and H. H. Hu, Director of the Fan Memorial Institute of Biology, both of whom have a wide knowledge of the living plants of China. They soon realized that it represented no known living tree, and comparisons showed that the cones and leafy shoots were essentially the same as those described by Miki from fossil specimens in Japan. Additional collecting by C. J. Hsueh in 1946 resulted in the discovery of several additional trees, and greatly augmented the material available for study. During the fall of 1947, C. T. Hwa, also a student of Professor Cheng, spent several months in the region, with financial support provided by the Arnold Arboretum of Harvard University through the cooperation of E. D. Merrill,¹⁷ and with a small grant from the University of California.

In the region to the south, in western Hupeh, Hwa found several hundred additional trees of *Metasequoia*, made a comprehensive collection of the woody plants of this general area, and brought out a large quantity of viable seeds. With adequate material for study at hand, Hu and Cheng have recently published an account of this new tree under the name *M. glyptostrobooides*.¹⁸ In an earlier paper, Hu¹⁹ had mentioned the discovery of this living tree in his brief discussion of another fossil species, *S. chinensis*, which had been described by Endo from the Oligocene flora of Fushun, Manchuria;²⁰ Hu correctly transferred it to the genus *Metasequoia* on the basis of its stalked, decussate-scaled cones. The leaves and cones of the living trees of *Metasequoia*, as described by Hu and Cheng, differ in no essential respects from those of the fossils on which Miki based his generic description. But there are four additional characters, of particular interest to paleobotanists, which are mentioned by these authors: (1) "opposite, distichously arranged foliage shoots"; (2) "leaves—opposite"; (3) "staminate flowers axillary and terminal, opposite, on racemose or paniculate flowering branchlet-system"; (4) cone-bearing twigs "with decussate linear leaves before maturity, and with conspicuous leaf scars and with persistent bracts at the base in winter." This paper by Hu and Cheng, describing the occurrence and external characters of *M. glyptostrobooides*, represents a major contribution to the botany of China and of the world.

With this significant information regarding *Metasequoia*, both fossil and living, coming over from Japan and China, I have reexamined with graduate students at the University of California²¹ the abundant conifer material in our Tertiary collections from the western United States, and later the collections at the United States National Museum. We have also studied the descriptions and illustrations of Heer and others, covering material from Cretaceous and Tertiary horizons elsewhere in the northern hemisphere. All of the specimens previously referred to *S. heerii*, and many of those assigned to *S. langsdorffii* and several other fossil species, have the cone or foliage characters of *Metasequoia*. In addition, staminate aments like those of the living species have been found in the Bridge Creek flora (Oligocene) of Oregon, and the Elko flora (Miocene) of Nevada, in association with typical cones and leafy twigs of *Metasequoia*. These fossil aments and twigs from Nevada had previously been referred to *Taxodium*.²² It has become increasingly apparent that many of the specimens assigned to *Sequoia* and *Taxodium* from the Cretaceous and Tertiary floras of North America are properly referable to *Metasequoia*. A generic revision of some of these is now in preparation, under the auspices of the American Philosophical Society and the Carnegie Institution of Washington. At this time it seems desirable to rewrite the description of *Metasequoia* on the basis of the paleobotanical evidence. In this description

and the following discussion the data provided by fossil specimens will be the primary consideration, though in some cases our knowledge of corresponding parts of the living tree has enabled us better to interpret them. All consideration of fossil stems is here omitted, since study of the wood of *Metasequoia*, modern and fossil, has not progressed to a point where significant distinguishing characters between it and *Sequoia* have been noted.

Genus Metasequoia Miki.—*Description.*—Foliage shoots straight, decussate; alternate pairs which come out from top and bottom of branch are twisted approximately 90° into horizontal plane of laterally disposed pairs so that all lie along one plane on branch, in opposite pairs; pairs originating in vertical plane often missing and represented by scars preserved on top surface of branch; diverging at angles approaching 90° , commonly ranging in length up to 8 cm., deciduous. Needles decussate, twisted with their decurrent bases to form two oppositely paired ranks, closely spaced, diverging at angles approaching 90° in typical, mature specimens; up to 1.8 cm. long, averaging about 8 mm., longest on proximal half of shoot and gradually reduced in length toward its tip; up to 2 mm. in width; obtusely tipped, narrowed and twisted at point of attachment to decurrent base; midrib well defined, with stomata parallel to it on ventral surface. Staminate cones ovate, up to 5 mm. long, with decussate bracts; closely spaced, sessile and decussate on elongate spikes; rotated into one plane so that they appear to be attached in opposite pairs. Ovulate cones globose to ovoid or elliptic, up to 2 cm. long and 1.5 cm. wide, averaging 1.5 by 1.2 cm. (at one American locality (Mollala) they are globose, 1.6 to 2.4 cm. in diameter, open cones slightly broader); cone-scales 12 to 24, decussate, peltate on broad-based stalks, discs lenticular to hexagonal, with transverse, medial grooves. Seeds about 3 mm. long, 1 mm. wide, the surrounding wings asymmetrical, notched at the apex, with over-all length of 4 to 5 mm., width of 3 to 3.5 mm. Cones terminal on stout, straight or curved shoots; cone-bearing shoots probably lateral, up to 4 cm. long and 2 mm. in diameter, more slender at the middle, bearing scaly leaves at base in complete specimens, rarely with scaly leaves or leaf-scars preserved along the shoot, probably widely spaced and evidently decussate.

Discussion.—It is unusual to find leafy shoots attached to branches in the fossil record. Their position is decussate in every instance, though the shoots that come out at right angles to the plane of the branch are commonly missing, and may be represented only by scars which show on the exposed surface of the slab. These fossil shoots average somewhat longer than those of the living *M. glyptostroboides* which we have examined. Some specimens, especially those from Elko, Nevada, and Mollala, Oregon, both of Miocene age, have slender, curving shoots with needles more

openly spaced and directed distally at higher angles than the typical specimens from Bridge Creek and other localities; studies of foliage of living trees have not progressed to a point where we can determine whether there is corresponding variation in *M. glyptostroboides*. Differences of opinion have been expressed regarding the mode of attachment of the needles. I agree with Stebbins²³ that their position is decussate; this may be readily observed at the tips of young shoots on seedlings which we have growing in Berkeley. The older shoots on herbarium specimens, as well as the leafy shoots preserved as impressions on our fossils, also show clearly the alternating position of attachment of successive pairs of needles and the twisting of their decurrent bases to bring all the pairs of needles into a single plane. Thomas Morley, graduate student in botany at the University of California, has at my suggestion sectioned the stem of a leafy shoot of the living *M. glyptostroboides* (sheet No. 753389, Univ. Calif. Herbarium). He finds the leaf gaps opposite at each node, with each successive pair at right angles to the pair below. However, in alternating nodes the leaf gaps and the decurrent leaf bases are twisted approximately 90°, which brings all the points of attachment of the needles into essentially the same plane along the shoot. An original decussate arrangement is thus confirmed by Morley's slides. No difficulty has been experienced in recognizing the opposite position of the needles in well-preserved fossils of *Metasequoia*; under low magnification the relations of needles and leaf bases to the shoot are as clear as with modern material; both living and fossil specimens are distinguishable at a glance from leafy shoots of *Sequoia* and *Taxodium* in which the needles are alternately disposed along the shoots. Like the pairs of needles, the decussately attached staminate cones are twisted into a single plane along the twig so that they are disposed in opposite or nearly opposite pairs. The staminate cones of a fossil specimen from the Oligocene Bridge Creek flora of Oregon are much smaller than those from Elko, and appear to represent a less-developed stage; in the living species there is a similar range in size. A specimen from Elko shows six spikes, in close association like those of the living species. The considerable range in size and shape of the ovulate cones, and in the numbers of their cone-scales, is no wider than is to be found in cones of the living species, and we have no present basis for determining whether such variation will provide criteria for recognizing two or more species.

As stated in the description, needles are commonly lacking from the cone-bearing shoots, and even their attachment scars are difficult to make out on most fossil specimens. The scars are readily visible on shoots of the living plant, and needles may remain in attachment while the cone is on the tree. When it has fallen, the needles are shed; the fossil record is made up exclusively of cones which have fallen to the ground, and the presence of needles on their shoots is not to be expected. Since this dis-

cussion deals with fossil material, in which the cones and leafy shoots are not commonly attached to branches, we have had to qualify certain of our statements regarding their mode of attachment. However, there is little doubt that this follows the general pattern of the living *M. glyptostrobooides*, in which the vegetative and reproductive shoots, and their needles and scales, are prevailingly decussate.

A survey of the characters of *Metasequoia* as seen in fossil material, and a comparison of these characters with those of *Sequoia* and *Taxodium*, provides the following bases for distinguishing this Chinese genus:

(1) Leafy shoots decussate, disposed distichously in opposite pairs along the branches in *Metasequoia*; spiral, disposed distichously and alternately in *Sequoia* and *Taxodium*.

(2) Leafy shoots deciduous in *Metasequoia* and *Taxodium*; remaining on the branches 3 to 4 years in *Sequoia*.

(3) Needles decussate on the shoots of *Metasequoia*, twisted and disposed distichously in opposite pairs; spirally disposed, commonly distichous, on the shoots of *Sequoia* and *Taxodium*.

(4) Stomata in parallel bands on each side of the midrib in *Metasequoia* and *Sequoia*; irregularly transverse in *Taxodium*.

(5) Staminate cones decussate on spikes, twisted into distichous, opposite pairs, in *Metasequoia*; spirally disposed on spikes in *Taxodium*; solitary in the axils of terminal needles in *Sequoia*.

(6) Pistillate cones terminal on elongate, probably lateral, leafy shoots in *Metasequoia* (they are lateral in the living species), the needles widely spaced, deciduous, leaving a naked cone-shoot on shedding; terminal on short scaly shoots which develop at the ends of leafy shoots of the preceding year in *Sequoia*; cones at the ends of branches in *Taxodium*.

(7) Cone scales decussate in *Metasequoia*; spirally disposed in *Sequoia* and *Taxodium*.

Our conclusion that *Metasequoia* rather than *Sequoia* was the dominant conifer of the Arcto-Tertiary Flora, whose southward migration from high latitudes has been so well established,^{6, 7} calls for reconsideration of the paleoecology and floristics of the Tertiary period. It is of primary significance to note that it was not an evergreen but a deciduous conifer which ranged northward to latitude 82° in Grinnell Land during the Eocene. The deciduous habit of *Metasequoia* was wholly consistent with that of the majority of its angiosperm associates in the older Tertiary floras from high northern latitudes. This same group of trees, only slightly altered in composition, is widely known at middle latitudes, both in North America and Asia, during middle Tertiary time. The need for determining the associates of the living *M. glyptostrobooides* becomes at once apparent, for they may represent a closer approach to the Arcto-Tertiary Flora than any modern vegetation as yet studied. It is necessary also to learn as much

as possible about the modern environment of *Metasequoia*, to serve as a guide for the reconstruction of its habitats in past ages.

In February and March, 1948, with Dr. Milton Silverman, Science Writer for the San Francisco *Chronicle*, I made a brief trip to western Szechuan and eastern Hupeh under the auspices of the Save-the-Redwoods League, and with the cordial coöperation of Chinese scientists and of Chinese and American officials. It was our good fortune to have accompanying us C. T. Hwa who had previously collected extensively in this area. The redwoods of China live in valley bottoms and slopes at altitudes from 4000 to 4450 feet. The discovery trees at Mo-tao-chi, Szechuan, including the largest tree observed²⁴ with a diameter of 64 inches above the buttress and a height of 98 feet, are growing in the midst of rice-paddies, and no other trees occur within 100 yards. But in the valley of Shui-hsa-pa, in western Hupeh, scores of *Metasequoias* were observed not only on the borders of rice-paddies on the floodplain, but extending up ravines under conditions which appeared to be relatively natural.²⁵ This is in no sense a forest, for trees occur largely in isolated ravines in association with other conifers and with deciduous hardwoods which show every evidence of being second growth. These associates had been widely noted at corresponding elevations during our 5-day trip into the area, and the plant formation has been described by Cheng²⁶ as occurring between 400 and 2000 meters elevation in this area. Among the more common hardwoods noted in immediate association with *Metasequoia* are chestnuts (*Castanea henryi* and two other species), a small-leaved oak (*Quercus glandulosa*), sweet gum (*Liquidambar formosuna*), and cherry (*Prunus*) of an undetermined species. An evergreen shrub related to our spice-bush (*Lindera*) is one of the most abundant members of the understory. On adjacent higher slopes the birch (*Betula luminifera*) and the beech (*Fagus longipetiolata*) are common, and in one ravine there is a large tree of katsura (*Cercidiphyllum japonicum*, var. *chinensis*). Four evergreen conifers, *Cunninghamia lanceolata*, *Cephalotaxus fortunei*, *Pinus massoniana* and *Taxus chinensis*, are of regular occurrence here, and small fan palms (*Trachycarpus fortunei*) were noted at various places in the Shui-hsa-pa area. This assemblage is essentially the same as that recorded in the fossil record of the Eocene from high latitudes; many of the figured specimens of *Metasequoia* from Greenland and Alaska have on the same slabs leaf impressions of katsura or birch; the Oligocene and Miocene floras from the John Day Basin of Oregon and other localities in the western United States include all of the angiosperm genera (except the palm) above recorded, in association with fossils of *Metasequoia*. Numerous genera which are common members of the Arcto-Tertiary Flora, both in the Eocene of high latitudes, and in middle Tertiary deposits at middle latitudes, have not yet been noted in immediate association with *Metasequoia*.

at Shui-hsa-pa; however, our field work was of limited duration and carried on at a season unfavorable for easy recognition of deciduous trees. We already know that species of *Alnus*, *Acer*, *Carpinus*, *Ostrya* and *Ulmus* have been observed within a few miles of *Metasequoia* trees, and it is probable that other genera known in the fossil record will be recorded during the field season of 1948 while Professor Cheng and his associates are working in this region.

Prior to the study of the trees of this region, the nearest surviving equivalents of the Arcto-Tertiary Flora which I have seen are the mixed bald cypress (*Taxodium*)—hardwood forest of the Wabash river valley in Illinois and Indiana, and the hardwood forests with associated conifers at middle altitudes on the Island of Hondo in Japan. The occurrence in the Shui-hsa-pa region of *Metasequoia* and *Cercidiphyllum* now restricted to Asia,²⁷ together with many angiosperm genera which occur today in North America as well, gives these groves in the ravines of western Hupeh a closer resemblance to the Eocene floras of Greenland, Spitzbergen and Alaska, and to the Oligocene-Miocene floras of Oregon and Manchuria than any living group of plants known to me. Not only has *Metasequoia* come down through the ages to survive in Central China, but the whole assemblage of which it is a part has had a long geologic history, and has participated in wide migrations. During these movements covering thousands of miles, and continuing for millions of years, some genera have disappeared and others have been added; minor changes in leaf or fruiting characters which are the basis for specific distinctions have appeared; but the Arcto-Tertiary Flora as we know it from early Tertiary rocks at high northern latitudes is so fully represented in the *Metasequoia* groves of Central China that there can be no question as to the holarctic origin of this modern vegetation.

That being the case, we may turn to this region for suggestions regarding the physical environment occupied by the Arcto-Tertiary Flora at many localities in the northern hemisphere during past ages. The nearest station which provides climatic data is Chungking, 140 miles to the southwest, and at an elevation more than 3000 feet lower. Annual precipitation here (43-year record) is 43.1 inches, of which 2.3 inches fall in the winter, 11.1 inches in the spring, 17.7 inches in the summer, and 12 inches in the autumn. The mean annual temperature (44-year record) is 66.2°F., ranging from a low of 48.6° in January to a high of 84.4° in August; the mean monthly minimum (6-year record) ranges from 35° in January to 70° in August, and the extreme lowest recorded temperature in 25 years is 28.9°; the mean monthly maximum (6-year record) ranges from 59° in January to 104° in August, and the extreme highest recorded temperature in 25 years is 111°. Relative humidity averages about 82% (16-year record), and there is little monthly variation. It seems probable that at

Shui-hsa-pa, 4000 feet in elevation, there is higher rainfall and a wider range in temperature. At the time of my visit in March, temperatures were mild and light rains fell daily. Villagers reported that there is no snow or frost, and the general aspect of the understory, made up of evergreen shrubs in the Lauraceae, Theaceae, Euphorbiaceae and Palmae, is suggestive of winter temperatures which rarely fall below freezing. Discussing the climate of the Red Basin of Szechuan, on the eastern rim of which our area is located, Cressey has stated:²⁸ "The climate is temperate and mild. Despite the location in the interior of the continent, it is protected from extremes of temperature by the surrounding mountains, and the contrast between summer and winter is not great. Summer temperatures seldom exceed 100°F., while during the winter the thermometer does not usually drop below freezing."

The only vegetation in North America which is closely comparable with *Metasequoia* and its associates of Central China is the bald cypress-hardwood forest of the south Atlantic coastal plain and the Gulf of Mexico,

TABLE 1
SHOWING PRECIPITATION AND TEMPERATURE IN REGIONS OCCUPIED BY *METASEQUOIA*,
TAXODIUM AND *SEQUOIA*

	ALTI- TUDINE, FEET	WINTER	PRECIPITATION, INCHES				FARRENHEIT TEMPERATURE		
			SPRING	SUMMER	AUTUMN	ANNUAL	MEAN ANNUAL	EXTREME MAX.	MIN.
Chungking	754	2.3	11.1	17.7	12.0	43.1	66.2	111	28.9
Brunswick, Ga.	14	9.3	9.8	18.6	12.0	49.7	68.4	103	13.0
Glennville, Ga.	175	9.7	9.9	17.4	9.9	46.9	67.1	106	11.0
Hammond, La.	44	14.9	14.0	18.1	11.2	58.5	67.5	106	1.0
Eureka, Calif.	62	19.5	10.2	1.0	8.3	39.0	51.6	85	20.0

extending northward up the Mississippi Valley to Illinois and Indiana. This is a lowland forest, reaching a maximum altitude of about 475 feet in the valley of the Wabash River. At latitudes corresponding to Shui-hsa-pa in Louisiana and Georgia, this forest lives within a few tens of feet of sea level, ranging up to about 200 feet in Georgia. As in Central China, precipitation is well distributed and adequate over all this region (table 1), with more than half falling in the spring and summer months. In the southeastern United States at the latitude of Shui-hsa-pa, temperatures show essentially the same mean average, but the extreme minima are much lower.

The bald cypress, *T. distichum*, of the American forest has its best development in swamps and on swamp borders, but its occurrence with hardwoods on moist floodplains more closely resembles the environment of *Metasequoia* in Central China. All of the angiosperm associates in the groves at Shui-hsa-pa have been noted with the exception of *Cercidiphyllum* and *Trachycarpus* which are confined to Asia; *Sabal* takes the

place of the latter genus in the southern United States. In spite of their geographic separation and marked topographic differences, the general aspect of the *Taxodium*-hardwood forest is surprisingly similar to that of the *Metasequoia*-hardwood assemblage. This resemblance is the more significant since during middle Tertiary time *Taxodium* and *Metasequoia* lived together in western North America and in northeastern Asia, in association with the same angiosperms; during early Tertiary time, these two conifers were widely distributed at high latitudes and with the same genera of hardwoods. *Taxodium* is almost as widely distributed in the Arcto-Tertiary Flora as *Metasequoia*. We conclude that this forest type had its origin at the north, in Eocene and Cretaceous time, in a region with a summer-wet and winter-cool climate; the deciduous habit of all the dominant trees, including *Metasequoia* and *Taxodium*, is consistent with such a climate. By Oligocene and Miocene time, a similar forest was widely established as far south as Oregon and Nevada, and down into Japan and Manchuria. By the end of the Miocene, *Metasequoia* appears to have disappeared from this continent; with a changing climatic regime in western North America, the surviving *Taxodium*-hardwood forest has been confined to southeastern North America. In Asia *Taxodium* is rare or absent in Pliocene floras; here *Metasequoia* has continued down to the present, though it is now confined to a limited area where environmental factors are favorable. This tree is reproducing on a limited scale, but its continued existence will be determined by the pressure put upon it by a people whose land, fuel and timber resources are wholly inadequate. A conservation committee has recently been organized in Nanking, and we may hope for its success in protecting some of the groves in which *Metasequoia* and its associates are living. *Glyptostrobus*, a close relative of *Taxodium*, has survived in the lowlands of South China. *Sequoia*, the living genus most similar to *Metasequoia*, is likewise a relict genus, restricted to the California and Oregon coast. Here the rainfall regime is wholly different from that in Central China, though the temperature, somewhat lower at this more northerly latitude, resembles that of western Szechuan in its equability (table 1). Our present knowledge of the Tertiary floras of Europe indicate that Heer was correct in his original reference of material from the Miocene of Switzerland to this genus, and that *Sequoia* rather than *Metasequoia* lived on this continent during the Tertiary period. Wide-spread submergence in western Europe at this time appears to have provided living conditions which favored *Sequoia*, although *Metasequoia* may be found in the fossil record of the Tertiary interior, farther to the east.

Having in mind the deciduous habit of *Metasequoia* and *Taxodium*, the deciduous hardwood associates of these genera, both in living forests and in the fossil record, and the modern environments in Central China and southeastern North America, we may suggest certain physical conditions

which may have been best suited to the origin and development of the Arcto-Tertiary Flora. A summer-wet climate appears to have been the primary requisite, with a total annual rainfall in excess of 40 inches. Moderate temperature seldom falling below freezing is also indicated, though a winter characterized by lower temperatures is indicated by the prevailing deciduous habit. The apparent absence of polar ice-caps during at least the early part of the Tertiary period, and postulated ocean circulation, makes it reasonable to accept the occurrence of *Metasequoia* and its associates as far north as Grinnell Land; possibly it was the darkness of the arctic night rather than low winter temperatures which brought about the annual shedding of the leaves of these trees, a habit which has been retained in *Metasequoia* even though winter temperature in its habitat in Central China seems not to make the deciduous habit a modern necessity. It is doubtful whether the present altitude of the *Metasequoia* occurrence, at about 4000 feet, has always been an essential requirement. More probable is the assumption that the high valleys occupied by these trees provide the only existing habitat which is suitable. Farther to the north at lower altitudes, extremes of temperature or precipitation characterize the climate of China, and although the fossil record shows that *Metasequoia* has lived there in the past, it can no longer do so. The limited area in eastern Szechuan and western Hupeh where the trees are known to have survived is surrounded by mountain ranges which protect them from the climatic extremes which characterize other inland environments. An equable climate rather than a high altitude appears to be the determining factor in the modern occurrence of *Metasequoia*.

We may summarize our current knowledge of the fossil occurrence and history of the genus *Metasequoia* as follows:

(1) Many of the fossil leaves and cones from the Cretaceous and Tertiary rocks of high northern latitudes, and from middle latitudes in North America and Asia, which have previously been referred to *Sequoia* and *Taxodium*, are now known to be *Metasequoia* on the basis of recent descriptions of fossil and living members of this genus in Asia.

(2) Vegetative and reproductive units in the fossil record differ in no essential respect from those of the living *M. glyptostroboides* of Central China. Like those of this tree, they are characterized by the decussate arrangement of their shoots, leaves and scales, and by the deciduous habit of leafy and cone-bearing shoots.

(3) Occurrence of *Metasequoia* in the Arcto-Tertiary Flora, at high latitudes in the Eocene epoch, at middle latitudes in the Oligocene and Miocene, provides evidence of the northern origin of this Flora, and of its southward migration during the Tertiary period.

(4) Survival of *Metasequoia* in Central China, in association with many of the hardwood genera recorded with it in the fossil records of the

northern hemisphere, provides a basis for reconstructing the climatic conditions under which the Arcto-Tertiary Flora had its origin and subsequent migration. A humid climate with summer rainfall, and with moderate temperatures not regularly falling below freezing, may be suggested as best suited to this deciduous forest during its past history.

¹ Endlicher, S., *Syn. Conif.*, 197-198 (1847).

² Brongniart, A., *Prodrome Hist. Veg. Foss.*, 108 (1828).

³ Heer, O., *Flora Tert. Helv.*, 1, 54, pl. 20, f. 2; pl. 21, f. 4 (1855).

⁴ Heer, O., *Flora Foss. Arct.*, 7 vols. (1868-1883).

⁵ Lesquereux, L., *U. S. Geol. Surv. Terr.*, VII, 76 (1878).

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⁷ Chaney, R. W., *Ecol. Mon.*, 17, 144-146 (1947).

⁸ Lesquereux, L., *U. S. Geol. Surv. Terr.*, VII, 77, pl. 7, f. 13 (1878).

⁹ Heer, O., *Flora Foss. Arct.*, 2, 3, 36, pl. 4, f. 4a (1870).

¹⁰ Heer, O., *Ibid.*, 2, 4, 464, pl. 43, f. 1 (1869).

¹¹ Heer, O., *Ibid.*, 3, 3, 5, pl. 2, f. 8 (1874).

¹² Ashley, J. F., in an unpublished report, written in 1938 when a graduate student, he suggested that stalked cones from Elko might be placed in a genus intermediate between *Sequoia* and *Taxodium*.

¹³ Endo, S., *Proc. Imper. Acad. (Tokyo)*, 12, 172, f. 5, 7-13 (1936).

¹⁴ Miki, S., *Jap. J. Bot.*, 11, 261-263, f. 8 (1941).

¹⁵ Sargent, C. S., *Manual of Trees of North America*, p. 894 (1922).

¹⁶ Heer, O., *Flora Foss. Arct.*, 4, 1, 63, pl. 12, f. 2a; pl. 13, f. 9-11 (1876).

¹⁷ Merrill, E. D., *Arnoldia*, 8, 1-8 (1948).

¹⁸ Hu, H. H., and Cheng, W. C., *Bull. Fan. Mem. Inst. Biol.*, 1, 153-161, pl. 1 (1948).

¹⁹ Hu, H. H., *Bull. Geol. Soc. China*, 26, 105-107 (1946).

²⁰ Endo, S., *Jap. J. Geol. and Geog.*, 6, 27-29, pl. 7 (1928).

²¹ Acknowledgment is due T. R. Pray and R. H. Shan for their assistance.

²² Lesquereux, L., op. cit., 73, pl. 6, f. 13.

²³ Stebbins, G. L., *Science*, 108, 95-99 (1948).

²⁴ Hwa reports seeing a tree more than 7 feet in diameter and over a hundred feet tall near Shu-hwi-chang, Hupeh.

²⁵ It is not possible to determine, in our present state of knowledge, that any of the *Metasequoia* trees are truly native. However, their association with the same conifers and angiosperms at several places strongly indicates that they have a natural place in this plant formation.

²⁶ Cheng, W. C., *Trav. For. Toulouse*, 1, 150 (1939).

²⁷ *Ginkgo biloba* is also living here, but was noted only near villages and is probably not native.

²⁸ Cressey, G. B., *China's Geographic Foundations*, pp. 312-313 (1934).

INFLUENCE OF AMINO ACIDS ON GROWTH OF *DATURA* EMBRYOS IN CULTURE*

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Investigations of the growth of *Datura* embryos in culture have shown that as early as the preheart stage, the embryos can be excised from the seeds and grown in an artificial medium containing only the major inorganic salts and sucrose. Such cultured embryos sometimes showed premature differentiation of root or plumule and grew to the seedling stage in less time than is required for the normal cycle of capsule ripening and seed germination. However, initial growth of the excised embryos, from preheart to mature embryo, never equalled that of the corresponding period of development in the seed which covers from 10 to 14 days (Sanders^{12, 13}). Addition of certain vitamins and organic acids did not markedly affect growth. However, addition of some organic complexes, notably coconut milk and malt extract, definitely increased the growth rate and favored normal development of the embryos (van Overbeek, Conklin and Blakeslee¹⁶; Blakeslee and Satina¹).

Studies on the nitrogen metabolism of higher plants have shown that many plants grown in sand culture, especially if deprived of inorganic nitrogen, can absorb and utilize certain sources of organic nitrogen. Legumes have been found better able to use organic nitrogen than other plants, such as wheat and barley (Virtanen and Linkola¹⁷). Plants furnished with external supplies of different amino acids have been found to have distinct morphological variations (Virtanen and Linkola¹⁷; Steinberg¹⁸). Tomato and tobacco seedlings deprived of inorganic sulphur were found able to use DL-methionine as a source of sulphur (Miller⁹). Though many amino acids can be utilized, toxicity is frequent and may be due either to specific acids or to unfavorable concentrations. Under controlled optimum conditions for growth, ammonium nitrogen has been shown to be utilized more rapidly than nitrate, but under usual conditions for growth, nitrates have proved to be the best external source of nitrogen for higher plants (Nightingale^{9, 10}).

With the development of tissue culture techniques, it has been possible to show that plant tissues, organs and embryos can assimilate inorganic nitrogen. On the whole, nitrates have again proved to be the best source of nitrogen although ammonium salts have been found available and, in some cases, a good source. For the most part, nitrogenous organic compounds have been toxic to plant tissue cultures, but there are exceptions. With tomato roots, White¹⁹ found that glycine with thiamin satisfactorily

replaced yeast extract in the nutrient. For growth of bacteria-free crown-gall tissue of sunflower, Riker and Gutsche¹¹ found urea to be the only organic nitrogenous compound approaching the effectiveness of nitrate. Some of the amino acids (DL-alanine, L-arginine HCl, L and DL-aspartic acid and L-glutamic acid) did prove to be available for growth and were not toxic when present with nitrate. In the culture of orchid embryos, addition of amino acids to a medium containing nitrate failed to improve growth and frequently showed toxicity (Withner²⁰; Spoerl¹⁴). Spoerl found that arginine and aspartic acid under certain conditions were as effective as ammonium nitrate, but used in addition to ammonium nitrate, they showed no stimulation of growth. He found that the effect of the amino acids depended upon concentration, light conditions, species of orchid and age of embryos. In studying the problem of rooting in asparagus stem tips in artificial culture, Galston⁴ found that glutamine and arginine stimulated growth but were ineffective for root formation. Kent and Brink⁶ observed that casein hydrolysate,† an amino acid complex, prolonged embryonic growth and inhibited germination of *Hordeum* embryos *in vitro*. Although the majority of amino acids supplied to plant cultures have proved to be growth inhibitors, alanine, arginine, aspartic acid, glutamic acid and glycine have proved beneficial to growth under certain conditions.

Materials and Methods.—Plants used in these investigations were *Datura innoxia* (S.I. 115) and *D. stramonium* (S.I. 1) (from the collection of Dr. A. F. Blakeslee) and were grown under greenhouse conditions. Self embryos were selected at the pre- or early heart stage (longitudinal axis, 0.1 to 0.25 mm.), dissected out of the seeds and grown in artificial liquid nutrients. Ten embryos per nutrient were used. Experiments ran for twelve days and results for individual media are expressed as growth values, final average length/original average length.

Basal media consisted of water, major inorganic salts, trace elements, vitamins and sucrose. The water used was double-distilled in Pyrex glass. The major salt formula was composed to obtain the following proportions of ions, 8 K : 1 Ca : 1 Mg and 4 NO₃ : 3 PO₄ : 3 SO₄. The following salts were used with "1" representing 1 millimolecular weight per liter of solution: 2 KNO₃, 1 Ca(NO₃)₂, 2 KH₂PO₄, 1 NaH₂PO₄, 1 MgSO₄ and 2 K₂SO₄. In some of the experiments, the inorganic nitrogen was increased so that the ratios were 8 K : 1 Ca : 1 Mg and 6 NO₃ : 3 PO₄ : 1 SO₄, and the formula became 4 KNO₃, 1 Ca(NO₃)₂, 3 KH₂PO₄, 1 MgSO₄ and 1 KCl. Increased nitrate, however, did not affect the growth values to any extent. The former formula gave growth values of 6.21 for *D. innoxia* and 5.5 for *D. stramonium*; the latter, 6.45 for *D. innoxia* and 4.88 for *D. stramonium*. Trace elements provided were in p. p. m.: B, 0.1; Mn, 0.1; Zn, 0.3; Cu, 0.1; Mo, 0.1; Fe, 0.5. Vitamin concentrations were in p. p. m.:

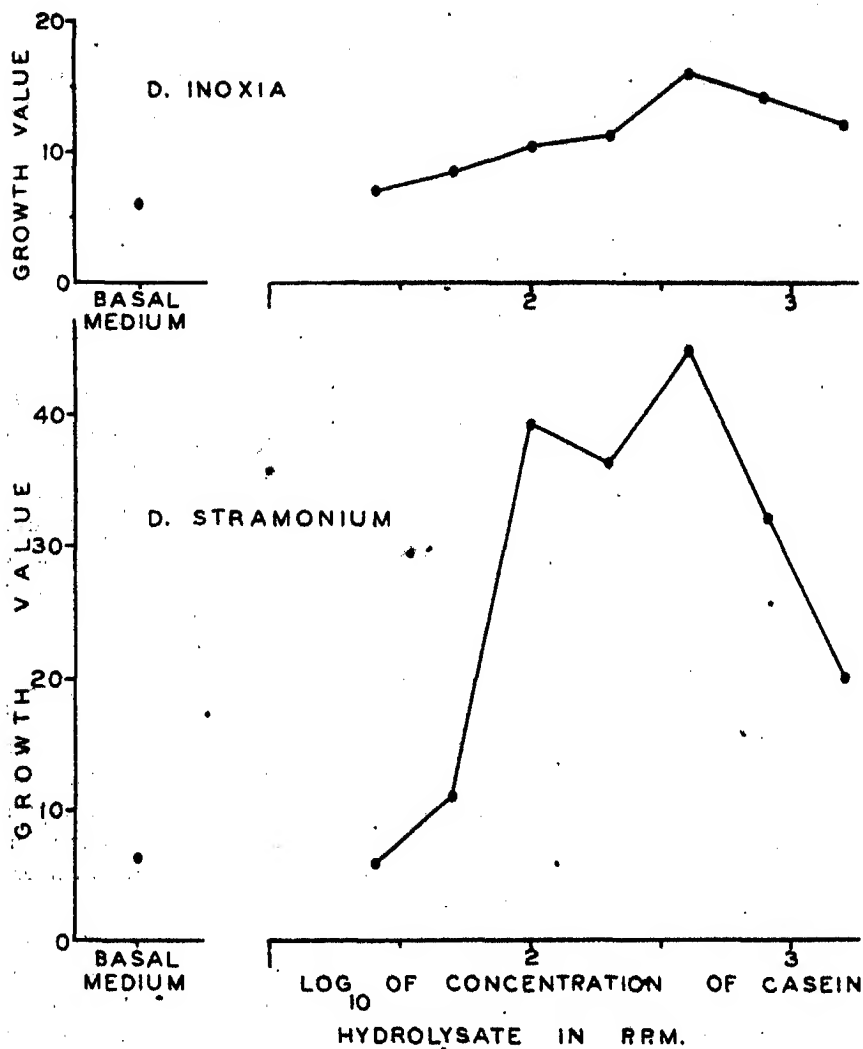


FIGURE 1

Response of preheart embryos of two species of *Datura* to casein hydrolysate with cysteine and tryptophane added to the basal medium.

thiamin HCl, 0.1; pyridoxine HCl, 0.8; niacin, 0.8. Sucrose was used at 2 per cent for *D. innoxia* and 4 per cent for *D. stramonium* (Sanders¹²). All nutrients were adjusted to between pH 5.7 and 6.0.

Response of Embryos to Casein Hydrolysate with Cysteine and Tryptophane Added to the Basal Medium.—Casein hydrolysate together with cysteine and tryptophane (each at 6.67 mg. per 100 mg. casein) was added to the

basal medium at 0, 25, 50, 100, 200, 400, 800 and 1600 p. p. m. Growth values for this series are represented in the graphs of figure 1. Growth of embryos of both species increased markedly especially in the range 100 to 800 p. p. m. Embryos of *D. stramonium* were much more stimulated than those of *D. innoxia*. At 400 p. p. m., the concentration which gave best growth in both species, growth values were 44.8 and 16.1, respectively.

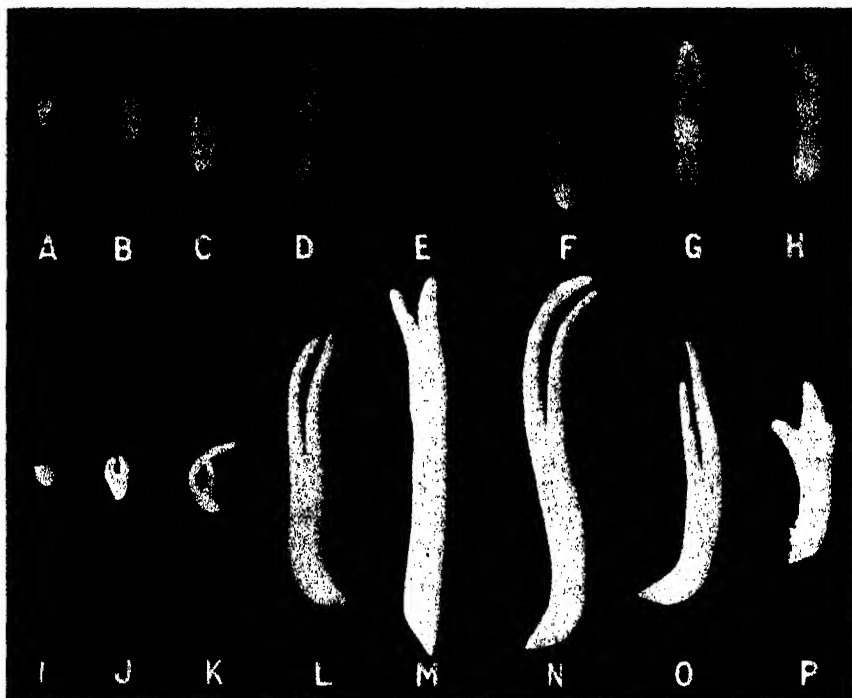


FIGURE 2

Photographs ($\times 54$) of *Datura* embryos grown for twelve days in a nutrient series to which casein hydrolysate with cysteine and tryptophane was added. A-H, embryos of *D. innoxia*. I-P, embryos of *D. stramonium*. A and I, in basal medium; B and J, in basal medium plus casein hydrolysate at 25 p. p. m.; C and K, at 50 p. p. m.; D and L, at 100 p. p. m.; E and M, at 200 p. p. m.; F and N, at 400 p. p. m.; G and O, at 800 p. p. m.; H and P, at 1600 p. p. m.

At 25 and 50 p. p. m., growth values were not much larger than for the basal medium, and at 800 and 1600 p. p. m., they decreased markedly in *D. stramonium* and somewhat in *D. innoxia*. Representative embryos from these series are shown in figure 2. The amino acid complex increased embryonic differentiation as well as the actual size of the embryos. After twelve days in basal medium, embryos were approximately 1 mm. in length. Embryos grown to this size in the seed have arrived at the late heart or

early torpedo stage and have well-defined cotyledons and no root primordia (Sanders¹²). In basal medium, *D. innoxia* embryos showed retarded cotyledonary development and frequently premature root primordia, and *D. stramonium* embryos showed little differentiation although cotyledon primordia were sometimes discernible (figure 2, A and I). With addition of casein hydrolysate, even at the lower concentrations, embryonic differentiation became more normal. Throughout the *D. innoxia* series, cotyledons were shorter in relation to the hypocotyl than are cotyledons of embryos grown in the seed. Abnormalities and root primordia appeared when casein hydrolysate was present, but less frequently and on much larger embryos than in basal medium.

Response of Embryos to Mixtures of Amino Acids and to Ten Single Amino Acids Added to Basal Medium.—In an attempt to identify the growth factors in casein hydrolysate, embryos were grown in a series of nutrients

TABLE 1

COMPOSITION OF AMINO ACID MIXTURES ADDED TO BASAL MEDIUM USED FOR GROWTH OF *Datura* EMBRYOS. (ACIDS MARKED WITH AN ASTERISK WERE THE COMPONENTS OF "MODIFIED MIXTURES" A AND B)

MIXTURE A		MIXTURE B	
Amino Acid	Concentration, p. p. m.	Amino Acid	Concentration p. p. m.
DL-alanine	6.0	*L-cystine	1.5
*L-arginine	12.0	*L-histidine	9.0
*L-aspartic acid	18.0	L-hydroxyproline	1.5
L-cysteine	20.0	DL-isoleucine	15.0
L-glutamic acid	60.0	L-leucine	15.0
Glycine	1.5	*DL-methionine	9.0
*L-lysine	18.0	*DL-serine	18.0
DL-phenylalanine	12.0	DL-threonine	12.0
*L-proline	24.0	L-tryptophane	20.0
L-tyrosine	18.0	*DL-valine	24.0

containing mixtures of amino acids or individual amino acids. Three controls were run for the series, basal medium, basal medium with NH_4Cl at 108 p. p. m. (an amount roughly equivalent to the amino nitrogen in casein hydrolysate at 300 p. p. m.), and basal medium plus casein hydrolysate at 300 p. p. m. with L-cysteine and L-tryptophane. Concentrations of individual amino acids (with the exception of cysteine and tryptophane which were used at the previous concentrations given above), were based on the amino acid analysis of casein hydrolysate (Harrow⁶). Amounts approximately equivalent to the content of the acids in hydrolyzed casein at 300 p. p. m. were determined. The amino acids were divided into two groups of ten each; mixture A included those acids which have been found beneficial for plant growth under certain conditions (Burkholder, Castle and Smith⁸; Riker and Gutsche¹¹; Spoerl¹⁴; White¹⁹); mixture B, the remaining ten acids which have shown no effect or have been

inhibitory to growth. The composition of mixtures A and B are given in table 1. Acids marked with an asterisk are those which were included in modified mixtures A and B. Amino acids of mixture A were also added singly to the basal medium at the same concentrations as in the mixture. Data from this series of nutrients are represented by the graphs in figure 3. In both species, addition of ammonium chloride increased growth somewhat suggesting an ammonium salt might be beneficial in the basal medium, but it did not approach the stimulatory effect of casein hydrolysate. Addition of mixtures A and B together in the same medium closely reproduced the results obtained with casein hydrolysate, strongly suggesting that the growth stimulus is due to the amino acids present in hydrolysed

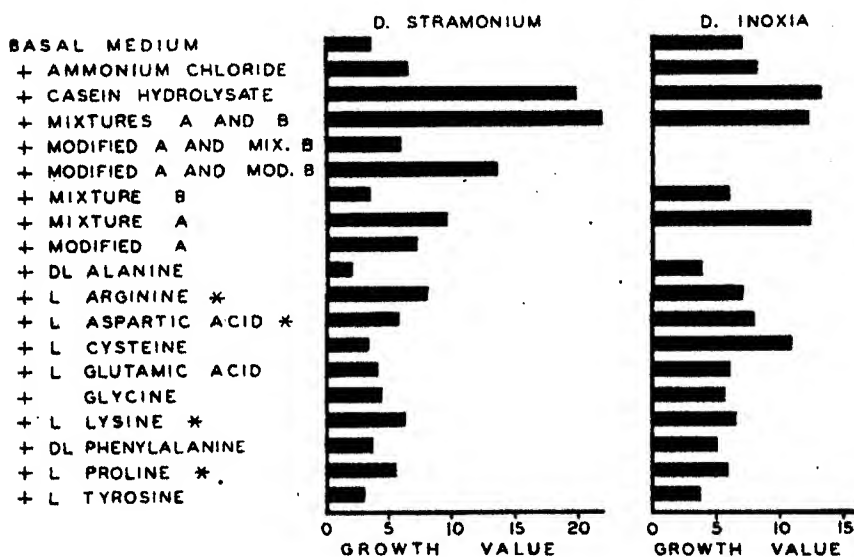


FIGURE 3

Response of preheart embryos of two species of *Datura* to ammonium chloride and various organic nitrogen sources added to the basal medium.

casein. Addition of B alone gave no increase in growth. Addition of A alone gave a growth value for *D. innoxia* embryos equal to that for A and B, suggesting that the entire stimulatory effect of casein hydrolysate might be contributed by the amino acids in A. The growth value for *D. stramonium* embryos on basal medium with A alone was less than half that with A and B suggesting that an interaction of the acids in the two mixtures is necessary to produce the growth stimulus obtained with casein hydrolysate. Addition of single amino acids also gave different results for the two species. With *D. innoxia* embryos, cysteine alone gave a definite increase in growth; aspartic acid showed a slight increase; alanine, phenylalanine, tyrosine

and perhaps glycine showed inhibition. With *D. stramonium* embryos, arginine, aspartic acid, lysine and proline increased growth; alanine and perhaps tyrosine showed inhibition. Modified mixture A was made up of the four acids stimulatory to *D. stramonium* embryos when added separately. Comparison of the growth value for modified A with that for A indicates that at least some of the six omitted acids were functional in promoting growth either individually or by interaction with other acids. Mixture B added with modified A decreased rather than increased embryo growth. Omission of six acids from A has apparently upset the amino acid balance which favored growth. Modified mixture B was made up by omitting five of the acids in B which have shown inhibition in other investigations. Modified B added to the basal medium with modified A almost doubled the growth value for modified A alone. This group of nine acids provided a more favorable balance. By careful selection and combination it might be possible to reproduce the growth stimulus of the original 20 acids by a balanced mixture of fewer acids. Embryos of *D. innoxia* were grown on basal medium plus aspartic acid and cysteine, the two acids which gave increased growth for this species, but the growth value was less than for either of the acids alone. Balance of amino acids is apparently an important factor determining the influence of these compounds upon growth of *Datura* embryos.

Amino acids which individually stimulated growth, four for *D. stramonium* and two for *D. innoxia*, were also tested at three concentrations each; arginine at 5, 10 and 20 p. p. m.; aspartic acid, cysteine, lysine and proline at 10, 20 and 40 p. p. m. Growth values for *D. stramonium* embryos increased from 3.3 to 6.8 with changes in arginine concentration from 5 to 20 p. p. m., decreased from 10.0 to 6.5 with changes in lysine concentration from 10 to 40 p. p. m., and were fairly constant with the changes in the concentrations of aspartic acid and proline (4.4 to 4.8 with the former and 6.1 to 5.6 for the latter). Growth values for *D. innoxia* embryos were 6.9, 8.9 and 6.9 with aspartic acid at 10, 20 and 40 p. p. m., and increased from 5.0 to 13.7 with cysteine from 10 to 40 p. p. m. Interaction of the acids casts some doubt on the possibility of determining optimum concentrations for growth by addition of varying amounts of single acids. This procedure does provide some evidence concerning the effect of different concentrations of the individual amino acids on growth and differentiation.

It has been mentioned that addition of casein hydrolysate to the basal medium favored normal differentiation of the embryos in culture. Mixtures A and B together were equally beneficial to normal differentiation. The other mixtures and the single amino acids were less effective for normal differentiation, but certain characteristics appeared. Although growth values for *D. innoxia* embryos in basal medium with A plus B and with A alone were very similar, the patterns of growth differed. With A plus B,

cotyledons of the embryos were short in proportion to the hypocotyls as with casein hydrolysate (figure 2, B-H). With A alone, hypocotyls were short in proportion to cotyledons. Addition of mixture A resulted in the same type of growth in *D. stramonium* embryos whereas with A plus B, cotyledon-hypocotyl proportions were normal as in figure 2, L-O. Modified A had the same effect as A, but modified A plus modified B produced embryos with proportionally short cotyledons. Differentiation as well as growth is influenced by the interaction of the amino acids present. Some single amino acids had recognizable effects on type of growth. Alanine interfered with cotyledon and hypocotyl differentiation in *D. innoxia* embryos but allowed formation of premature root primordia and even slight growth in the region of the plumule. Cysteine at 20 and 40 p. p. m. increased the number of root primordia and actual root growth in *D. innoxia* embryos. Lysine, especially at 10 p. p. m., had a similar effect on *D. stramonium* embryos. It also contributed to the formation of translucent, watery tissue instead of the firm, white tissue of healthy embryos beyond the heart stage. Changes in the balance of amino acids can apparently enhance premature differentiation of embryos to seedlings as well as prevent it.

Discussion.—Most plants naturally assimilate inorganic nitrogen, and amino acids are generally poor sources of nitrogen for plants in sand cultures and for many plant cultures *in vitro*. The metabolism of plants and of plant parts which synthesize their own organic nitrogenous compounds is apparently upset by supplying an excess of these substances. To study the effects of amino acids on growth in the presence of inorganic nitrogen, other than inhibition or abnormalities, it is necessary to culture plant tissues, organs or embryos which are able to use to advantage an external source of amino acids in addition to their own capacity for synthesis. Sunflower tissue (Riker and Gutsche¹¹) and orchid embryos (Withner²⁰; Spoerl¹⁴) with an adequate supply of inorganic nitrogen are apparently able to synthesize their necessary amino acids as rapidly as they can use them. Asparagus stem tips (Galston⁴), tomato root tips (White¹⁹), immature *Hordeum* embryos (Kent and Brink⁶) and immature *Datura* embryos can apparently use amino acids faster than some of these compounds can be synthesized in such isolated structures. It may well be that *in vivo* amino acids are supplied to such structures by other parts of the plant. Without an external source of amino acids, young *Datura* embryos grown in culture require a much longer time to mature than in the seed or with a mixture of amino acids in the nutrient.

The behavior of mixtures indicates that the influence of these acids on growth results from interaction rather than accumulation of the individual effects of single acids. Evidence of such interaction has been found with numerous microorganisms. For example, Bonner² demonstrated in a

single gene mutant of *Neurospora* that a block in the conversion of keto isoleucine to isoleucine resulted in an accumulation of the former which inhibited the conversion of keto valine to valine, thus causing a deficiency of two amino acids. Inhibition of growth in *Lactobacillus arabinosus* by glycine can be counteracted by the addition of D-alanine (Kobayashi, Fling and Fox⁷). Inhibition of growth in *Streptococcus bovis* by isoleucine, leucine, threonine or norleucine can be counteracted by valine, glutamic acid, methionine or cystine. Inhibition caused by equal amounts of phenylalanine and tyrosine, which singly had no effect, can be counteracted by tryptophane, glutamic acid or cystine. It is suggested that inhibiting amino acids may block the synthesis of another acid and that the counteracting acid may be the acid blocked or an acid from which it can be formed (Washburn and Niven¹⁸).

Single additions of amino acids have been found to have characteristic effects on the morphology of plants. This phenomenon suggests that the balance of amino acids probably changes during development and that such changes may influence growth and differentiation. Steinberg¹⁸ suggests changes in the balance of amino acids as the basis for the production of morphological symptoms of certain plant diseases. Some of the problems of differentiation, relative growth and dormancy, would appear to be closely related to amino acid metabolism.

The effect on embryo growth of casein hydrolysate and of the mixture of amino acids approximating the composition of casein hydrolysate is similar to the beneficial effects of other organic complexes, namely coconut milk and malt, which have been found to stimulate growth of *Datura* embryos, favor embryonic differentiation and retard premature germination (van Overbeek, Conklin and Blakeslee¹⁶; Blakeslee and Satina¹). The varied activities of such natural complexes may well be due to their component amino acids.

Differences in the effects of amino acid mixtures and of single acids on embryos of two separate species suggest that the amino acid content of female parent tissues and of endosperm may be important in interspecific crosses, especially crosses in which embryos are first formed but later abort. An attempt to correlate similarity of amino acid requirements of self embryos of the parent species and of the hybrid embryos with species crossability might prove more successful than earlier attempts to correlate species crossability with similarity of sucrose and inorganic salt requirements of the embryos (Sanders¹⁸).

Summary.—Early heart embryos of *Datura innoxia* and *D. stramonium* (0.1 to 0.25 mm. in length) were dissected out of the seeds and grown in artificial culture in basal medium alone and also with additions of casein hydrolysate, mixtures of amino acids, and single amino acids.

Casein hydrolysate with cysteine and tryptophane markedly improved growth and embryonic differentiation in the range 100 to 800 p. p. m.

A mixture of 20 amino acids approximating their occurrence in casein hydrolysate at 300 p. p. m. resulted in growth equal to that with casein hydrolysate indicating that the amino acids are responsible for the growth stimulus provided by hydrolyzed casein.

Differences in growth values for lesser mixtures and for individual amino acids, which were for the most part well below those for the mixture of 20 acids, provide evidence that the growth stimulus of the combination of 20 acids results from physiological interactions rather than from the summation of effects of individual acids.

Characteristic patterns of differentiation appeared with some mixtures and with some individual acids.

The species differed in their responses both to mixtures and to single amino acids.

Balance of amino acids appears to be a controlling influence on both growth and differentiation of plant embryos.

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‡ From General Biochemicals, Inc., Chagrin Falls, Ohio.

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ON THE STABILITY AND INSTABILITY OF SHOCK WAVES

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Communicated August 6, 1948

The following communication contains a criterion for the stability and instability of plane stationary shock waves (compression shocks) attached to the vertex V of a pointed obstacle O in a uniform field of flow.¹ Behind the shock line the flow may be rotational and non-isentropic; viscosity and thermal conductivity are assumed zero in this theory. It will be assumed that the shock line and boundary of the obstacle are analytic

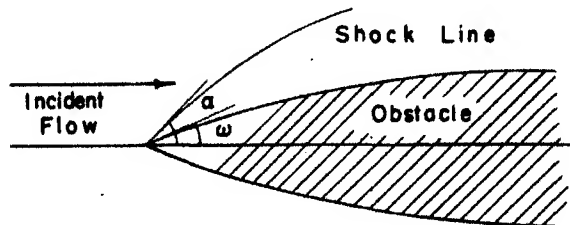


FIGURE 1

curves and that the functions u_α (velocity components), p (pressure) and ρ (density) defining the flow are analytic in the region behind, and also along the shock line.

Angles α and ω give the inclination of the shock line and obstacle at V relative to the direction of the incident flow. Attention may be confined in the following discussion to the "upper half" of the shock line as indicated in the figure. The range of permissible values of α is given by $\alpha_0(M) < \alpha \leq 90^\circ$ where $M(>1)$ is the mach number of the incident flow and $\alpha_0(M)$ is the value of $\sin^{-1} 1/M$ in the first quadrant. Denote by s the arc length along the shock line, measured toward V , and by η the arc length along the obstacle measured in the direction of flow. Let K and κ be the curvatures of the contour of the obstacle and shock line, respectively, and put $K_{(n)} = d^n K/d\eta^n$, $\kappa_{(n)} = d^n \kappa/ds^n$ for $n = 0, 1, 2, 3, \dots$ so that $K_{(0)} = K$ and $\kappa_{(0)} = \kappa$. It can be shown that there exist relations of the form²

$$K_{(n)} = G_n(M, \alpha)\kappa_{(n)} + H_n, \quad n = 0, 1, 2, 3, \dots, \quad (1)$$

at point V , where the H_n are polynomials in κ and its derivatives with respect to s of order less than n . The quantities $G_n(M, \alpha)$ and the coefficients of the polynomials H_n are rational functions of M^2 with coefficients depending on the inclination α of the shock line. In particular for $n = 0$ the equation (1) becomes $K = G_0(M, \alpha)\kappa$. Actually the relations (1) hold at any point of the shock line when α is the inclination of the shock line and K the curvature of the stream line behind the shock line at this point but we shall be concerned primarily with these relations at the vertex V of the obstacle. For a given value of $M > 1$, a value of α for which any one of the functions $G_n(M, \alpha) = 0$ will be said to be singular relative to M .

The above relations (1) appear as *consistency conditions* for the shock wave solution of the hydrodynamical problem. If a shock wave solution exists for a singular value of α , corresponding to $G_i(M, \alpha) = 0$, then any variation in the value of $K_{(i)}$ will result in an inconsistency among the relations (1). Hence when α is singular there will exist in general no shock wave solution under the assumptions of continuity and differentiability involved in the derivation of the relation (1) for which the coefficient $G_i(M, \alpha)$ vanishes.

Denoting by m the mach number for the flow behind the shock line at V there exists a number $\beta = \beta(M)$ such that $\alpha_0(M) < \beta(M) < 90^\circ$ and such that $m = 1$ for $\alpha = \beta(M)$. Furthermore $m > 1$ for $\alpha_0(M) < \alpha < \beta(M)$ and $m < 1$ for $\beta(M) < \alpha \leq 90^\circ$. It can be shown that the zeros of the functions $G_n(M, \alpha)$, i.e., the singular shock angles α relative to any mach number $M > 1$, lie entirely within the interval $\beta(M) < \alpha \leq 90^\circ$ and are in fact dense in this interval.

The figure shows the nature of the graph of the angle ω for a given value of $M > 1$ and the location of the singular shock interval. Point A corresponds to the value $\alpha = \alpha_0(M)$ and B to the value $\alpha = \beta(M)$ at which $m = 1$. In the open interval AB no singular shock angles α occur, nor is there a singular shock angle at the end-point B . The singular shock

angles are dense in the open interval BD and a singular angle occurs at D . The ω curve reaches its maximum height at a point E , corresponding to a value of α , represented by the point C , in the singular shock interval.³

The following criterion for the stability and instability of attached shock waves is based on the above stated results. Let \bar{O} be an obstacle, analogous to the obstacle O , having inclination $\bar{\omega}$, curvature \bar{K} and derivatives of curvature $\bar{K}_{(n)}$ at its vertex V . Then \bar{O} will be said to be in a δ neighborhood of O provided that $|\omega - \bar{\omega}| < \delta$ and $|K_{(n)} - \bar{K}_{(n)}| < \delta$ for $n = 0, 1, 2, 3, \dots$. Also the shock wave \bar{W} for the flow past the obstacle \bar{O} will be said to be in the ϵ neighborhood of the shock wave W for the flow past O when $|\alpha - \bar{\alpha}| < \epsilon$. On the basis of these definitions we now lay down the following definition of *local stability* or stability at the vertex V ; in this statement we are concerned only with the contour of the obstacle, the

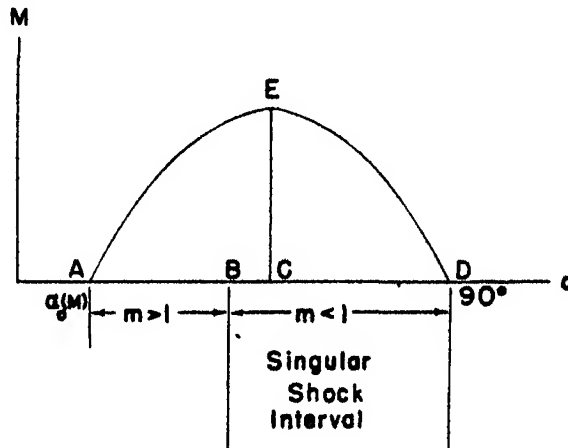


FIGURE 2

shock wave and the hydrodynamical solution in the immediate neighborhood of V . The shock wave attached to the vertex of the obstacle O , or the associated hydrodynamical solution, will be said to be *locally stable* or *stable* at V , relative to the incident flow, provided that for any $\epsilon > 0$ there can be found a $\delta > 0$ such that solutions with shock waves \bar{W} in the ϵ neighborhood of W exist for all obstacles \bar{O} in the δ neighborhood of O . Otherwise the shock wave attached to the vertex of the obstacle O (or the associated solution) will be said to be *locally unstable* or *unstable* at V .

Suppose that the shock angle α lies in the interval $\beta(M) \leq \alpha \leq 90^\circ$ for the flow past the obstacle O . Then if δ and ϵ are any two positive numbers we can find an inclination angle $\bar{\omega}$ and corresponding singular shock angle $\bar{\alpha}$ such that $|\omega - \bar{\omega}| < \delta$ and $|\alpha - \bar{\alpha}| < \epsilon$; this follows immediately from the fact that the singular values α are dense in the above interval and ω is a continuous function of α . Now consider the obstacle \bar{O} , with

vertex at V , having inclination $\bar{\omega}$ and for which $\bar{K}_{(n)} = K_{(n)}$ ($n = 0, 1, 2, 3, \dots$) at V . In general the set of relations (1), in which α is replaced by the above singular value $\bar{\alpha}$, will be inconsistent for the obstacle \bar{O} . If, however, these relations are consistent a slight variation in the value of $\bar{K}_{(0)}$, assuming $G_j(M, \bar{\alpha}) = 0$, while the values of all other $\bar{K}_{(n)}$ remain unchanged, will result in a new obstacle \bar{O} for which the relations (1) are inconsistent. Hence, there can be found an obstacle \bar{O} in any δ neighborhood of O for which no attached shock wave solution exists. *In other words a shock line for which the inclination α lies in the interval $\beta(M) \leq \alpha \leq 90^\circ$ is unstable at V (also unstable in the large from the following discussion).*

In the special case of flow past an infinite wedge two shock wave solutions are possible. Here the shock lines are straight lines and the flow behind the shock is uniform. Contrary to the general situation solutions now exist for all singular values of the shock angle α . However, those solutions for which the shock angle α lies within the interval $\beta(M) \leq \alpha \leq 90^\circ$, or, in other words, for which $m \leq 1$ (where m is the mach number for the flow behind the shock) will be unstable.

If the shock angle α lies in the interval $\alpha_0(M) < \alpha < \beta(M)$ so also will $\bar{\alpha}$ for $|\bar{\alpha} - \alpha| < \epsilon$ and ϵ sufficiently small. Corresponding to this value of ϵ we can find a δ such that for all inclination angles $\bar{\omega}$, for which $|\bar{\omega} - \omega| < \delta$, the associated shock angles $\bar{\alpha}$ will be in the interval $|\bar{\alpha} - \alpha| < \epsilon$. Hence for any obstacle \bar{O} , in the δ neighborhood of O , the associated shock angle $\bar{\alpha}$ will lie in the non-singular interval $|\bar{\alpha} - \alpha| < \epsilon$ and hence the formal power series determination, in the neighborhood of V , of the shock line and of the functions u_m , p and ρ behind the shock line will be possible. The proof of local stability requires only the convergence of these series for obstacles \bar{O} , with analytic contours, in the arbitrarily small δ neighborhood of the obstacle O for which the corresponding series are assumed to converge. Presumably these series will converge under this condition with the result that the shock line will be stable at V when the shock angle α lies within the interval $\alpha_0(M) < \alpha < \beta(M)$. It is not our intention, at present, to carry out the details of this convergence proof.

The concept of local stability has been introduced as a step toward the solution of the more proper, but also more complicated, problem of *stability in the large*. Conditions for stability in the large are to be phrased in an analogous manner except that we must here be concerned with the entire extent of the shock line, and solution of the hydrodynamical problem behind the shock line, including the conditions at infinity. In this connection it may be advisable to confine our attention in certain circumstances to a special class of obstacles, e.g., obstacles with analytic contours reaching to infinity and concave downward at all points of the portion of the contour under consideration, and so arrive at a concept of

stability relative to this special class. Moreover the problem can be extended, within the framework of our general method, by considering obstacles whose contours are composed of a finite number of analytic arcs with shock waves originating at their points of intersection.

It is evident that instability at V , or local instability, is sufficient to insure instability in the large. Hence, the above result on instability gives the complete answer to the problem of determining the conditions for instability of shock lines attached to the vertex V of an obstacle whose contour is an analytic curve. Since at most two shock angles α at V are mathematically possible the shock line which actually occurs and which corresponds to the shock line experimentally observed must therefore be the one whose inclination α lies in the interval $\alpha_0(M) < \alpha < \beta(M)$. This may be accepted as sufficient evidence for the stability (local or in the large) of shock lines with inclination α in the interval $\alpha_0(M) < \alpha < \beta(M)$ by those not interested in an existence-theoretic treatment of the problem.

¹ Prepared under Navy Contract N6onr-180, Task Order V, with Indiana University.

² The derivation of these relations and other results mentioned in this note are contained in several papers which we expect to publish later in the *Journal of Mathematics and Physics* under the following titles: "Calculation of the Curvatures of Attached Shock Waves"; "The Consistency Relations for Shock Waves"; and "The Distribution of Singular Shock Directions."

³ This conclusion is reached by an observation of the graphs of the functions ω and $-G_0(M, \alpha)$ shown in the paper "Calculation of the Curvatures of Attached Shock Waves."

PROGRESS IN THE STATISTICAL THEORY OF TURBULENCE*

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The fundamental notion of statistical mean values in fluid mechanics was first introduced by Reynolds. His most important contributions were the definition of the mean values for the so-called Reynolds' stresses and the recognition of the analogy between the transfer of momentum, heat and matter in the turbulent motion.

In the decades following Reynolds' discoveries, the turbulence theory was directed toward finding semi-empirical laws for the mean motion by methods loaned from the kinetic theory of gases. Prandtl's ideas on momentum transfer and Taylor's suggestions concerning vorticity transfer belonged to the most important contributions of this period. I believe that my formulation of the problem by the application of the similarity principle has the merit to be more general and independent of the methods

of the kinetic theory of gases. This theory led to the discovery of the logarithmic law of velocity distribution in shear motion for the case of homologous turbulence.

The next important step was the definition of isotropic turbulence by Taylor and the following period in the development of the theory of turbulence was devoted to the analysis of the quantities which are accessible to measurement in a wind tunnel stream. These quantities are the correlation functions and the spectral function. The general mathematical analysis of the correlations was executed by L. Howarth and myself. One has to consider five scalar functions $f(r)$, $g(r)$, $h(r)$, $k(r)$, $l(r)$. These functions determine all double and triple correlations between arbitrary velocity components observed at two points because of the tensorial character of the correlations. The two scalar functions for the double correlations are defined as follows:

$$f(r) = \frac{\overline{u_1(x_1, x_2, x_3)u_1(x_1 + r, x_2, x_3)}}{\overline{u_1^2}}, \quad (1)$$

$$g(r) = \frac{\overline{u_1(x_1, x_2, x_3)u_1(x_1, x_2 + r, x_3)}}{\overline{u_1^2}}.$$

Because of the continuity equation for incompressible fluids $g = f + \frac{r}{2} \frac{df}{dr}$.

For the same reason the triple correlations h , k and l can be expressed by one of them, e.g., by

$$h(r) = \frac{\overline{[u_1(x_1, x_2, x_3)]^2 u_1(x_1 + r, x_2, x_3)}}{[\overline{u_1^2}]^{3/2}} \quad (1a)$$

In addition we also deduced a differential equation from the Stokes-Navier Equation which gives the relation between the time derivative of the function f and the triple correlation function h .

$$\frac{\partial}{\partial t} (\overline{fu^2}) + 2[\overline{u^2}]^{1/2} \left(\frac{\partial h}{\partial r} + \frac{4}{r} h \right) = 2\nu \overline{u^2} \left(\frac{\partial^2 f}{\partial r^2} + \frac{4}{r} \frac{\partial f}{\partial r} \right). \quad (2)$$

We discussed this equation in two special cases:

(a) Small Reynolds number—in this case the triple correlations can be neglected and one obtains a self-preserving form for the double correlation function as a function of r/λ , where λ is defined by the relation

$$\frac{d\overline{u^2}}{dt} = -10\nu \frac{\overline{u^2}}{\lambda^2}. \quad (3)$$

(b) Large Reynolds number—in this case the terms containing the vis-

cosity can be neglected for not too small values of r and the functions f and h are assumed to be functions of the variable r/L ; L is a length characterizing the scale of turbulence. The hypothesis of self-preserving correlation function leads to the following special results. One can consider three simple cases:

1. $L = \text{constant}$; then we have $\overline{u^2} \sim t^{-2}$ (Taylor).
2. Loitziansky has shown that if the integral $\overline{u^2} \int_0^\infty r^4 f(r) dr$ exists, it must be independent of time, consequently $\overline{u^2} L^5 = \text{constant}$. Then $\overline{u^2} \sim t^{-5/3}$, $L \sim t^{1/3}$.
3. If the self-preserving character is extended to all values of r , i.e., also near $r = 0$, one has $\overline{u^2} \sim t^{-1}$, $L \sim t^{1/2}$ (Dryden).

On the other hand, Taylor introduced a spectral function for the energy passing through a fixed cross-section of a turbulent stream as the Fourier transform $\mathfrak{F}_0(n)$ of the correlation function $f(r)$. The relation between \mathfrak{F} and f is given by the following equations:

$$\begin{aligned} f(r) &= \int_0^\infty \mathfrak{F}_0(n) \cos \frac{2\pi nr}{U} dn, \\ \mathfrak{F}_0(n) &= \frac{4\overline{u^2}}{U} \int_0^\infty f(r) \cos \frac{2\pi nr}{U} dr. \end{aligned} \quad (4)$$

In these equations n is the frequency of the fluctuation of the uniform velocity U as function of time. Relative to the stream, $\mathfrak{F}_0(n)$ can be replaced by $\mathfrak{F}_1(\kappa_1)$, where $\kappa_1 = \frac{2\pi n}{U}$, i.e., the wave number of the fluctuation, measured in the x_1 direction.

It is seen that in this period of the development of the turbulence theory the analytical and experimental means for the study of isotropic turbulence were clearly defined but (with the exception of the case of very small Reynolds numbers) no serious attempt was made to find the laws for the shapes of either the correlation or the spectral functions. I believe this is the principal aim of the period in which we find ourselves at present. Promising beginnings were made by Kolmogoroff, Onsager, Weizsäcker and Heisenberg. I do not want to follow the special arguments of these authors. I want rather to define the problem clearly and point out the relations between assumptions and results.

A—I will assume that the three components of the velocity in a homogeneous isotropic turbulent field, at any instant, can be developed in the manner of Fourier's integrals

$$u_i = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} Z_i(\kappa_1, \kappa_2, \kappa_3, t) e^{i(\kappa_1 x_1 + \kappa_2 x_2 + \kappa_3 x_3)} d\kappa_1 d\kappa_2 d\kappa_3. \quad (5)$$

B—The intensity of the turbulent field be characterized by the quad-

ratio mean value $\overline{u_i^2}$ level of the turbulence). Also there exists a function $\mathcal{F}(\kappa)$ such that $[\overline{u_i^2}]_0^t = \int^t \mathcal{F}(\kappa') d\kappa'$, where the symbol $[\overline{u_i^2}]_0^t$ means a partial mean value of the square of the velocity, the averaging process being restricted for such harmonic components whose wave numbers $\kappa_1, \kappa_2, \kappa_3$ satisfy the relation

$$\kappa_1^2 + \kappa_2^2 + \kappa_3^2 \leq \kappa^2. \quad (6)$$

If such a function exists it is connected with the spectral function of Taylor $\mathcal{F}_1(\kappa_1)$ by the relation

$$\mathcal{F}_1(\kappa_1) = \frac{1}{4} \int_{-\infty}^{\infty} \frac{1}{\kappa^3} \mathcal{F}(\kappa) (\kappa^2 - \kappa_1^2) d\kappa. \quad (7)$$

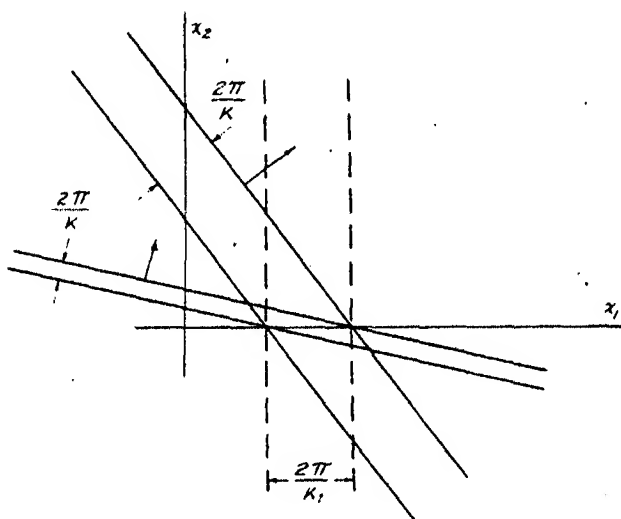


FIGURE 1

Contribution of oblique waves to plane waves in direction of x_1 .

This relation was found by Heisenberg. It expresses the geometrical fact that all oblique waves, figure 1, whose wave-length $\frac{2\pi}{\kappa} < \frac{2\pi}{\kappa_1}$, necessarily contribute in the one-dimensional analysis to the waves with wave length $2\pi/\kappa_1$.

C—It is evident that there must be an equation for the time derivative of $\mathcal{F}(\kappa)$ which corresponds to the equation for the time derivative of $f(r)$ which has been found by Howarth and myself. The physical meaning of this equation is evident. Let us start from the energy equation for a fluid element:

$$\frac{1}{2} \frac{\partial \overline{u_i^2}}{\partial t} + \overline{(u_i u_j + \delta_{ij} p / \rho) \frac{\partial u_i}{\partial x_j}} = \nu \overline{\frac{\partial^2 u_i}{\partial x_j^2} u_j}.$$

The right side represents the energy dissipation by viscous forces. The second term on the left side is the work of the Reynolds stresses; it represents a transfer of energy without actual dissipation. Our problem is to find $\partial \mathfrak{F} / \partial t$ by Fourier analysis and averaging process. One finds the contribution of the viscous forces to be equal to $-2\nu \mathfrak{F}(\kappa) \kappa^2$. Hence we write formally

$$\frac{\partial \mathfrak{F}}{\partial t} + \mathfrak{W}_\kappa = -2\nu \kappa^2 \mathfrak{F}(\kappa). \quad (8)$$

Here $\mathfrak{W}_\kappa d\kappa$ is the balance for the energy contained in harmonic components comprised in the interval $d\kappa$; obviously $\int_0^\infty \mathfrak{W}_\kappa d\kappa = 0$. C. C. Lin has shown that $\mathfrak{W}_\kappa = 2\mathcal{H}\kappa^2$, where

$$\mathcal{H}(\kappa) = 2(\kappa^2 \mathcal{H}_1''(\kappa) - \kappa \mathcal{H}_1'(\kappa))$$

and

$$\mathcal{H}_1(\kappa) = \frac{2(\bar{u}^2)^{1/2}}{\pi} \int_0^\infty h(r) \frac{\sin \kappa r}{\kappa} dr.$$

Unfortunately, this relation does not help, as far as the determination of f and h is concerned. For example, if one expresses h in terms of f from the Kármán-Howarth equation, calculates \mathfrak{W}_κ and substitutes the result in equation (8), one obtains an identity. It appears that at the present time one needs some additional physical assumption.

D—We assume that \mathfrak{W}_κ can be expressed in the form:

$$\mathfrak{W}_\kappa = \int_0^\infty \Theta \{ \mathfrak{F}(\kappa), \mathfrak{F}(\kappa'), \kappa, \kappa' \} d\kappa'. \quad (9)$$

The physical meaning of this assumption is the existence of a transition function for energy between the intervals $d\kappa$ and $d\kappa'$ which depends only on the energy density and the wave numbers of the two intervals. It follows from this definition that by interchanging κ and κ' , one has

$$\Theta \{ \mathfrak{F}(\kappa), \mathfrak{F}(\kappa'), \kappa, \kappa' \} = - \Theta \{ \mathfrak{F}(\kappa'), \mathfrak{F}(\kappa), \kappa', \kappa \}. \quad (10)$$

It must be noted that our assumption probably cannot be exact. It is very probable that the values of \mathfrak{F} for the difference and the sum of κ and κ' also enter in the transition function. I believe that the assumption gives a fair approximation when κ and κ' are very different, but it is certainly untrue if κ and κ' are nearly equal.

E—We furthermore specify the function Θ in the following way:

$$\Theta = -C \mathfrak{F}(\kappa)^{\alpha} \mathfrak{F}(\kappa')^{\alpha'} \kappa^{\beta} \kappa'^{\beta'}; \quad C = \text{const.} \quad (11)$$

It follows from dimensional reasoning that

$$\alpha + \alpha' = 3/2, \quad \beta + \beta' = 1/2.$$

As a result of the sequence of assumptions given above we obtain the equation:

$$\frac{\partial \mathfrak{F}}{\partial t} = C \left[\mathfrak{F}^{\alpha} \kappa^{\beta} \int_0^{\kappa} \mathfrak{F}(\kappa')^{1/2 - \alpha} \kappa'^{1/2 - \beta} d\kappa' - \mathfrak{F}^{1/2 - \alpha} \kappa^{1/2 - \beta} \int_{\kappa}^{\infty} \mathfrak{F}(\kappa')^{\alpha} \kappa'^{\beta} d\kappa' \right] - 2\nu \kappa^2 \mathfrak{F}. \quad (12)$$

Obviously if $\mathfrak{F}(\kappa)$ is known for $t = 0$, equation (12) determines the values of \mathfrak{F} for all times. If one neglects the first term on the left side, which represents the decay of turbulence and chooses the specific values $\alpha = 1/2$, $\beta = -3/2$ one arrives to the theory proposed by Heisenberg.

Let us consider the case of large Reynolds number but assume that κ is not so large that the term containing the viscosity coefficient becomes significant. Let us also assume that the first term on the left side is small by comparison to the second term. Physically this means that the energy entering in the interval $d\kappa$ is equal to the energy which leaves the interval. Then one has the relation:

$$\mathfrak{F}^{\alpha} \kappa^{\beta} \int_0^{\kappa} \mathfrak{F}(\kappa')^{1/2 - \alpha} \kappa'^{1/2 - \beta} d\kappa' = \mathfrak{F}^{1/2 - \alpha} \kappa^{1/2 - \beta} \int_{\kappa}^{\infty} \mathfrak{F}(\kappa')^{\alpha} \kappa'^{\beta} d\kappa'. \quad (13)$$

This equation is satisfied by the solution $\mathfrak{F}(\kappa) \sim \kappa^{-1/2}$, as one easily can see by substitution in 13. This result is independent, evidently, of the special choice of α and β . That is the reason why it was independently found by Onsager, Kolmogoroff and Weizsäcker. It is essentially a consequence of dimensional considerations. Let us now stay with the case of large Reynolds numbers by neglecting again the viscosity term but retaining the first term on the left side. In other words we consider the actual process of decay at large Reynolds numbers. Let us assume that \mathfrak{F} is a function of a non-dimensional variable κ/κ_0 , when κ_0 is a function of time. This assumption is equivalent to our former assumption that $f(\tau)$ is a function of τ/L ; i.e., we assume that \mathfrak{F} and f preserve their shapes during the decay. Evidently $\kappa_0 \sim 1/L$. Then the function \mathfrak{F} can be written in the form

$$\mathfrak{F}(\kappa) = \frac{\overline{u^2}}{\kappa_0} \Phi\left(\frac{\kappa}{\kappa_0}\right).$$

Then with $\frac{\kappa}{\kappa_0} = \xi$ and

$$\frac{\partial}{\partial t} \mathfrak{F} = \frac{\Phi}{\kappa_0} \frac{d\overline{u^2}}{dt} - \frac{\overline{u^2}}{\kappa_0^2} \Phi \frac{d\kappa_0}{dt} - \frac{\overline{u^2}}{\kappa_0^2} \Phi'(\xi) \xi \frac{d\kappa_0}{dt}$$

equation (12) becomes

$$\left(\frac{1}{\kappa_0} \frac{d\bar{u}^2}{dt} - \frac{\bar{u}^2}{\kappa_0^2} \frac{d\kappa_0}{dt} \right) \Phi - \frac{\bar{u}^2}{\kappa_0^2} \frac{d\kappa_0}{dt} \Phi' \xi + \mathcal{W}_\kappa = 0, \quad (14)$$

where

$$\mathcal{W}_\kappa = -C[\bar{u}^2]^{1/2} [\Phi^\alpha \xi^\beta \int_0^\xi \Phi(\xi')^{1/2-\alpha} \xi'^{1/2-\beta} d\xi' - \Phi^{1/2-\alpha} \xi^{1/2-\beta} \int_0^\infty \Phi(\xi')^\alpha \xi'^\beta d\xi'].$$

According to Loitziansky's results¹ $\frac{1}{\bar{u}^2} \frac{d\bar{u}^2}{dt} = \frac{5}{\kappa_0} \frac{d\kappa_0}{dt}$ and one obtains the equation

$$\xi^5 (\xi^{-4} \Phi)' = -5C \frac{[\bar{u}^2]^{1/2}}{\frac{d\bar{u}^2}{dt}} [\Phi^\alpha \xi^\beta I_0^\xi - \Phi^{1/2-\alpha} \xi^{1/2-\beta} I_\xi^\infty], \quad (15)$$

where

$$I_0^\xi = \int_0^\xi \Phi(\xi')^{1/2-\alpha} \xi'^{1/2-\beta} d\xi'; \quad I_\xi^\infty = \int_\xi^\infty \Phi(\xi')^\alpha \xi'^\beta d\xi'.$$

Let us assume that $4\alpha + \beta < 5/2$ as, for example, in the case of Heisenberg. Then for small values of ξ the right side of equation (15) is small in comparison with the term on the left side and one has

$$\Phi(\xi) \cong \text{const. } \xi^4.$$

If $4\alpha + \beta > 5/2$, \mathcal{F} begins with a lower power of κ than κ^4 and one can show that the integral $\int_0^\infty r^4 f(r) dr$ does not converge, so that Loitziansky's result is incorrect. I should like to investigate this second case in a later work. Let us assume, for the time being, that Loitziansky's result is correct and therefore the first case prevails. Then it follows that \mathcal{F} or Φ behaves as $(\kappa/\kappa_0)^4$ for small values of κ and is proportional to $(\kappa/\kappa_0)^{-4/5}$ for large values of κ . For any definite choice of α and β the differential equation (15) can be solved numerically. In June, 1947, I suggested to F. E. Marble that he carry out some such calculations and his results will be reported in a following publication. The result that $\mathcal{F} \cong \kappa^4$ for small values of κ was also found in a different way by C. C. Lin.

For the time being I propose an interpolation formula as follows:

$$\Phi(\xi) = \text{const. } \frac{\xi^4}{(1 + \xi^2)^{n/5}}. \quad (16)$$

This interpolation formula represents correctly $\Phi(\xi)$ for small and large values of ξ and has the advantage that all calculations can be carried out analytically by use of known functions. The results are as follows:

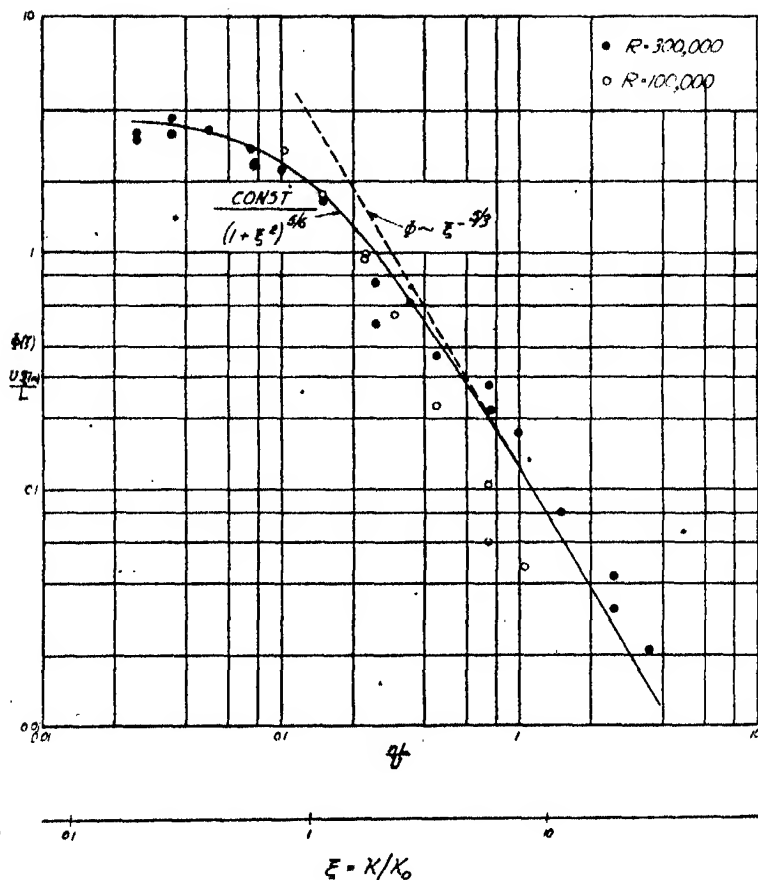


FIGURE 2

Comparison of observed and computed values of the frequency spectrum.

$$\begin{aligned}
 \mathcal{F}(\kappa/\kappa_0) &= \text{const.} \frac{(\kappa/\kappa_0)^4}{[1 + (\kappa/\kappa_0)^2]^{1/2}} \\
 \mathcal{F}_1(\kappa_1/\kappa_0) &= \text{const.} \frac{1}{[1 + (\kappa_1/\kappa_0)^2]^{1/2}} \\
 f(\kappa_0 r) &= \frac{2^{1/2}}{\Gamma(1/3)} (\kappa_0 r)^{1/2} K_{1/3}(\kappa_0 r) \\
 g(\kappa_0 r) &= \frac{2^{2/3}}{\Gamma(1/3)} (\kappa_0 r)^{1/2} \left[K_{1/3}(\kappa_0 r) - \frac{\kappa_0 r}{2} K_{-1/3}(\kappa_0 r) \right].
 \end{aligned} \tag{17}$$

The K 's are Bessel functions with imaginary argument. For small values of $\kappa_0 r$

$$f(\kappa_0 r) = 1 - \frac{\Gamma(2/s)}{\Gamma(4/s)} \left(\frac{\kappa_0 r}{2} \right)^{2/s} \quad (18)$$

as suggested by Kolmogoroff's theory.

I have compared these results with the measurements of Liepmann Laufer and Liepmann² carried out at the California Institute of Technology with the financial assistance of the N.A.C.A.† These observations were made in the 10-foot wind tunnel of the Guggenheim Aeronautical Laboratory using a grid whose mesh size was $M = 4$ inches. The measurements were made at a distance $x = 40.4M$ from the grid. Figure 2 shows the comparison of calculated and measured values for the spectral function

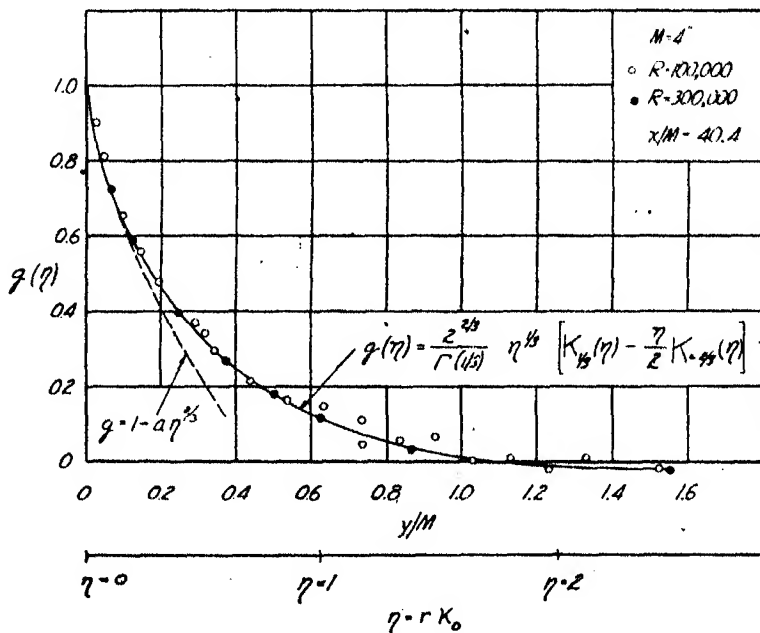


FIGURE 3

Comparison of observed and computed values of the correlation function g . The Reynolds number is based on the stream velocity and the mesh size.

$\mathcal{F}_1(\kappa_1)$. It has to be taken into account that the observed values of $\mathcal{F}_1(\kappa_1)$ have large scatter; the deviation for high values of κ_1 corresponds to the beginning influence of viscosity. Figure 3 gives a comparison between measured and calculated values of the correlation function $g(r)$. This function is chosen because the observations are more accurate than in any other case. It is seen that the agreement is almost too good in view of the assumptions made above. One must remark that there is only one arbitrary constant in the formula for g , viz., the constant κ_0 which deter-

mines the scale of the turbulence. It is true that some of the data of reference 2 do not show such a good agreement. The agreement is excellent for values of g larger than 0.1, but after that the measured values are higher than the calculated ones. Possibly some oscillations existing in the wind tunnel stream were interpreted as turbulence or the turbulence is not quite isotropic.

I believe that the merits of my deduction are: (a) the assumptions involved are exactly formulated; (b) the specific assumptions of Heisenberg's theory concerning the transition function are not used; (c) the actual process of decay is considered; (d) the analysis is extended to the lower end of the turbulence spectrum.

Concerning the case of large values of κ (small values of r) L. Kovásznyai³ introduced an interesting assumption which is more restricting than my assumption D . Obviously $\int_0^\kappa \mathcal{W}_\kappa d\kappa$ is the total energy transferred by the Reynolds stresses from the interval $(0 \rightarrow \kappa)$ to the interval $(\kappa \rightarrow \infty)$. Kovásznyai assumes—following Kolmogoroff's arguments—that this quantity is a function of $\mathfrak{F}(\kappa)$ and κ only. Then for dimensional reasons $\int_0^\kappa \mathcal{W}_\kappa d\kappa = \text{const. } \mathfrak{F}^{1/2} \kappa^{3/2}$. This assumption appears to be correct for large values of κ . When, however, the assumption is extended to the range of small values of κ and one substitutes \mathcal{W}_κ in equation (8) one can calculate easily $\mathfrak{F}(\kappa)$. Neglecting the viscous term, one obtains the relation

$$\mathfrak{F}(\kappa/\kappa_0) = \text{const.} \frac{(\kappa/\kappa_0)^4}{[1 + \mathfrak{F}^{1/2}(\kappa/\kappa_0)^{3/2}]^{17/2}} \quad (19)$$

The right side of equation (19) behaves as my corresponding equation (17) for small and large values of κ/κ_0 . It will be interesting to see how far the different transition from small to large values influences the accordance with observation.

* Presented at the Heat Transfer and Fluid Mechanics Institute, Los Angeles, California, June 23, 1948.

† The N.A.C.A. has kindly allowed presentation of these data prior to official N.A.C.A. publication.

¹ Loitziansky, L. G., "Some Basic Laws of Isotropic Turbulent Flow," Central Aero-Hydrodynamical Institute, Report No. 440, Moscow, 1939. Translated as N.A.C.A. Technical Memorandum 1079.

² Liepmann, H. W., Laufer, J., and Liepmann, K., "On Some Turbulence Measurements Behind Grids," Final Report N.A.C.A. Contract NAW 5442, July, 1948.

³ Kovásznyai, Leslie, S. G., "The Spectrum of Locally Isotropic Turbulence," *Phys. Rev.*, **73**, (9), 1115 (May, 1948).

NOTE ON THE LAW OF DECAY OF ISOTROPIC TURBULENCE

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Communicated by Theodore von Kármán, August 2, 1948

The law of decay of turbulence has been discussed by several authors by making various assumptions. Recently, Batchelor¹ made a summarizing discussion of some of these assumptions and their consequences. The present author has made a different assumption, which seems to be suggested by Kolmogoroff's concept of turbulence at high Reynolds numbers, and yet has not been covered by Batchelor's discussion. The result seems also to be in reasonably good agreement with all experimental data available. This result was originally obtained by a discussion of the spectrum, but a derivation using the correlation function itself is presented here.

According to Kolmogoroff, the double and triple correlation functions $f(r)$ and $h(r)$ for a distance r apart should be of the forms

$$u'^2\{1 - f(r)\} = v^2\beta_2\left(\frac{r}{\eta}\right), \quad (1)$$

$$u'^3h(r) = v^3\beta_3\left(\frac{r}{\eta}\right), \quad (2)$$

where β_2 and β_3 are definite functions of their argument $\xi = r/\eta$ for small values of ξ , at high Reynolds numbers, u' is the intensity of turbulence, and η and v are characteristic measures of length and velocity. Furthermore, η and v are given in terms of the rate of energy dissipation by the following relations

$$\eta = (\nu^3/\epsilon)^{1/4}, \quad v = (\nu\epsilon)^{1/4}, \quad \epsilon = -\frac{3}{2} \frac{du'^2}{dt}, \quad (3)$$

where t is the time, and ν is the kinematic viscosity coefficient. At the various stages of the decay process, ϵ changes. Comparing the fields at various stages of decay, one sees that $\beta_2(\xi)$ and $\beta_3(\xi)$ should be definite functions of ξ for small ξ in the course of time, i.e., there is self-preservation of $\beta_2(\xi)$ and $\beta_3(\xi)$.

We shall now show that if one makes the assumption of self-preservation of β_2 and β_3 , (3) follows as a consequence. Also, a definite law of decay is obtained which is in agreement with all experimental data so far available.

Substituting (1) and (2) into the equation of von Kármán and Howarth,

$$\frac{\partial}{\partial t}(u'^2f) + 2u'^3\left(\frac{\partial h}{\partial r} + \frac{4h}{r}\right) = 2\nu u'^2\left(\frac{\partial^2 f}{\partial r^2} + \frac{4}{r}\frac{\partial f}{\partial r}\right),$$

one has

$$\frac{du'^2}{dt} - \frac{dv^2}{dt} \beta_2(\xi) + v^2 \beta_2'(\xi) \xi \frac{1}{\eta} \frac{d\eta}{dt} + \frac{2v^2}{\eta} \left\{ \beta_2'(\xi) + \frac{4}{\xi} \beta_2(\xi) \right\} = \frac{2\nu v^2}{\eta^2} \left\{ \beta_2''(\xi) + \frac{4}{\xi} \beta_2'(\xi) \right\}.$$

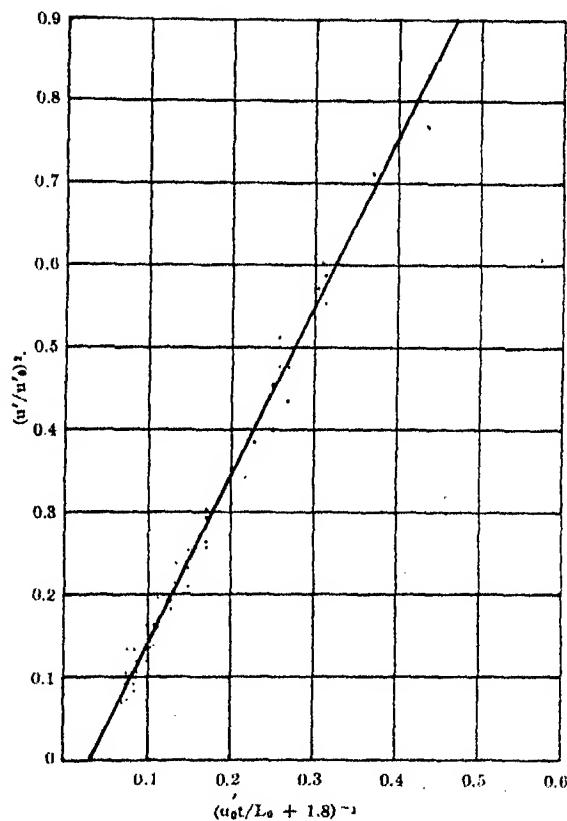


FIGURE 1

Comparison of the present law of decay with experiments.

Thus, the ratios

$$\epsilon : \frac{dv^2}{dt} : v^2 \frac{1}{\eta} \frac{d\eta}{dt} : \frac{v^2}{\eta} : \nu \frac{v^2}{\eta^2}$$

must be constants. From a consideration of the first, the fourth and the fifth terms, (3) follows at once. Including the other two terms, one obtains the additional relations:

$$v^2 = A(t - t_0)^{-1}, \quad \eta^2 = B\nu(t - t_0), \quad (4)$$

where A , B and t_0 are constants. From this, it can be easily seen that

$$\epsilon' = C(t - t_0)^{-2}, \quad (5)$$

and hence

$$u'^2 = \alpha(t - t_0)^{-1} + \beta, \quad (6)$$

where C , α and β are constants. This leads also to the relation

$$\lambda^2 = 10\nu(t - t_0)\{1 + (\beta/\alpha)(t - t_0)\}, \quad (7)$$

for the change of Taylor's microscale λ .

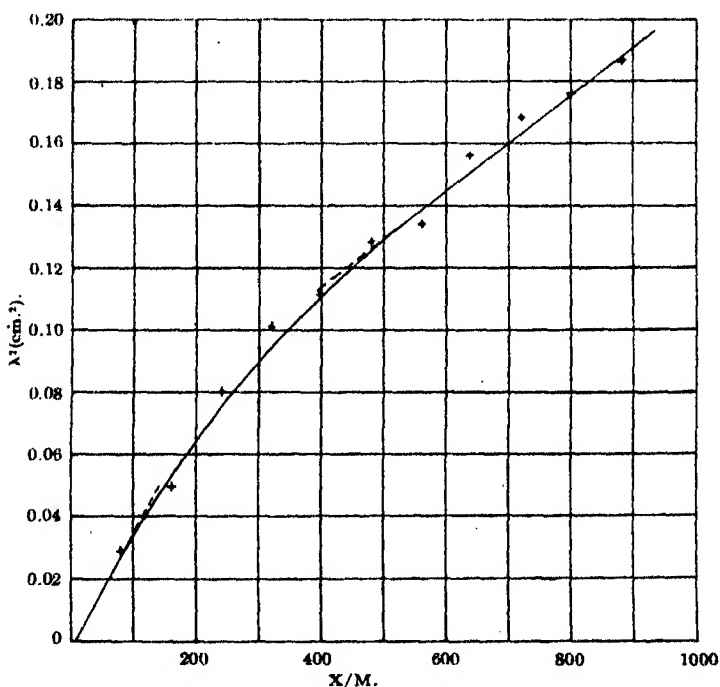


FIGURE 2

Comparison with experiments of the theoretical law of change of Taylor's microscale.

Notice that η^2 increases linearly in t . As η is the smallest scale, this diffusive nature is expected. Equations (5), (6) and (7) are merely consequences of this relation. Taylor's microscale λ does not in general possess the diffusive property any more (except when β is zero). But it can be easily verified that $R_\lambda = u'\lambda/\nu$ and $u'^{-2}R_\lambda$ changes linearly in time.

The law of decay just obtained is different from all previous ones. It

does however include the half-power law which is at least approximately satisfied by many of the experiments. On the other hand, Dryden's analysis² indicates that there are certain data which do depart from it. One can however take such results into account by the present law. The comparison is shown in figure 1, where one of Dryden's diagrams (von Kármán) is replotted according to the present scheme. This is the case departing most from the half-power law. It is seen that the agreement is good.

A more critical check is to compare the theory with directly measured values of λ , as λ depends on the derivative of u' . Such a comparison is made in figure 2. In making this comparison, the curve is supposed to begin with a slope of $10\nu M/U$ (M being mesh size of the grid, $U = x/t$) and approaches asymptotically to a straight line of slope $4\nu M/U$. These limiting straight lines are shown dotted, where they depart considerably from the experimental values. In fact, both the experimental points and these lines are reproduced from a figure kindly supplied to the author by Mr. Batchelor, based on experimental results of Dr. Townsend. The theoretical curve is then made to join up smoothly with the asymptotic straight line. The agreement is good. It may be noted that there is no freedom in this joining.

The decay process may thus be described as follows. At the beginning of the decay process, when the Reynolds number of turbulence is high, there is self-preservation in the sense of (1) and (2) for small r . As the Reynolds number decreases to very low values, the self-preservation property includes the whole $f(r)$ curve. This is the final stage of the decay process which has been considered by von Kármán and Howarth and investigated very much in detail recently by Batchelor and Townsend. The physical process is much clearer when presented in terms of spectrum. This discussion will be presented elsewhere.

The appearance of the arbitrary additive constant might seem to be a drawback in the theory. Actually, it is a necessity, if one follows the idea of Kolmogoroff. According to this theory, at high Reynolds numbers, the essential decay mechanism is governed by the high-frequency components only. On the other hand, the low-frequency components do contribute to the intensity. For example, if there is a superposed disturbance of low frequencies, the essential decay mechanism should not change. The detailed discussion of this point will be presented later in connection with a study of the energy spectrum. It might be noted that when $\beta = 0$, we have the half-power law of decay, and consequently a self-preservation of $f(r)$ itself. In any case, this is approximately satisfied for the initial stages of the decay process, when $t - t_0$ is small.

¹ Batchelor, G. K., *QuAM*, 6, 97-116 (1948).

² Dryden, H. L., *Ibid.*, 1, 28-30 (1943).

THE CYTOLOGICAL MECHANISM OF THE TRIPLOIDY-INDUCING EFFECT OF HEAT ON EGGS OF THE NEWT, *TRITURUS VIRIDESCENS*

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Communicated by E. G. Conklin, August 18, 1948

Extreme temperatures, both high and low, have been known for many years to induce polyploidy in plants. If the temperature shock is applied at the time of meiosis, diploid, and sometimes tetraploid, pollen grains are formed which, upon transfer to the styles of normal diploid plants, give rise to triploid (and, possibly, pentaploid) offspring. Treatment at a later time, during early stages of division of the zygote, may double the diploid chromosome complex and produce completely or partially tetraploid seedlings. As a rule, the percentage of polyploids resulting from temperature treatments is relatively low, ranging from less than 1% to about 13%.^{1, 2, 3}

Similar experiments have been carried out with eggs of a few species of animals. Since, at the time of laying or fertilization of the eggs of most animals, the maturation divisions are not yet completed, the abnormal temperature may interfere with the completion of these divisions and thus add one or more sets of maternal chromosomes to the diploid complement of the zygote. Refrigeration of eggs of a parthenogenetic race of the brine shrimp, *Artemia salina*, in some cases caused retention of the chromosomes usually eliminated in a polar body.^{4, 5} On the other hand, immersion of unfertilized eggs of the silk moth, *Bombyx mori*, in water at 46°C. for eighteen minutes resulted in the establishment of the diploid chromosome number through suppression of one of the maturation divisions. This treatment also induced the eggs to develop by parthenogenesis and fusion of diploid nuclei during cleavage produced tetraploid or partially tetraploid individuals.^{6, 7} In the greenhouse white fly, *Trialeurodes vaporariorum*, virgin females parthenogenetically produce haploid males exclusively as long as the temperature remains normal. Following exposure to temperatures ranging from 37 to 45°C., virgin females gave rise to about 10% diploid female offspring, presumably through suppression of the second maturation division.⁸

Among vertebrates, the polyploidizing effect of abnormal temperatures has been investigated extensively in various species of amphibians. Refrigeration of fertilized eggs for several hours, shortly after laying, produced a high percentage of triploid larvae in the newts, *Triturus viridescens*,^{9, 10} *Triturus pyrrhogaster*,^{11, 12} *Triturus similans* and *rivularis*,¹³ *Triton taeniatus*,¹⁴ and *Triton alpestris*,^{15, 16} and in the axolotl.¹⁷ In some

experiments with eggs of *Triturus viridescens*, all the surviving larvae were triploid. Treatment of eggs of *Triturus viridescens* at 36 to 37°C. for five to fifty minutes gave rise to 71% triploids among the surviving embryos^{18, 19} but was less effective with eggs of *Triturus pyrrhogaster*.^{19, 20} When eggs of the leopard frog, *Rana pipiens*, were exposed twenty minutes after insemination to 37°C. for four minutes, 38% developed into triploid embryos.²¹

As regards the mechanism responsible for the polyploidizing effect of extreme temperatures, earlier studies on plant and animal cells indicate that the change in temperature usually causes a breakdown of the spindle mechanism, either through direct action on the spindle fibers or through an effect on the centromeres or centrosomes, thus preventing the normal separation of the chromosomes at anaphase. In other cases the anaphase movements may be normal, but the two groups of chromosomes do not separate over a sufficient distance during telophase; so that a restitution nucleus is formed that incorporates all the chromosomes present in the original mitotic figure. In relatively small cells, a restitution nucleus may be formed in spite of normal telophase separation, if the abnormal temperature has no other effect than to prevent the division of the cell body.^{22, 23, 24}

In the eggs of amphibians with a diameter from one to two millimeters or more, the maturation spindle is extremely small in relation to the size of the cell as a whole and located at the surface near the animal pole. On the basis of the cytological studies mentioned above, it was assumed that cold or heat would bring about a suppression of the second polar body through a breakdown of the spindle and thus lead to the formation of a diploid egg nucleus.^{18, 19} This seemed to be a logical hypothesis since, at the time of laying of the amphibian egg, meiosis has progressed to metaphase of the second division but is blocked in this stage until fertilization occurs. The high percentage of triploid embryos produced by abnormal temperatures would thus be explainable by the fact that at the time of treatment the eggs always contain a mitotic figure that is arrested in metaphase, rather than an actively progressing mitosis. This reasoning was supported by the early observation that refrigeration of salamander eggs was no longer effective in inducing triploidy when the onset of the treatment was delayed until about thirty minutes after laying, at which time the second maturation division has progressed to a late anaphase.

Recently, Briggs²¹ has studied the changes in susceptibility to heat treatments of eggs of *Rana pipiens* during the first forty minutes after insemination. He finds that the percentage of triploids is highest (30%) when the treatment begins twenty minutes after insemination, and drops to zero at forty minutes. At twenty minutes, or shortly thereafter, the anaphase of the second maturation division is just beginning. Possibly, the heat

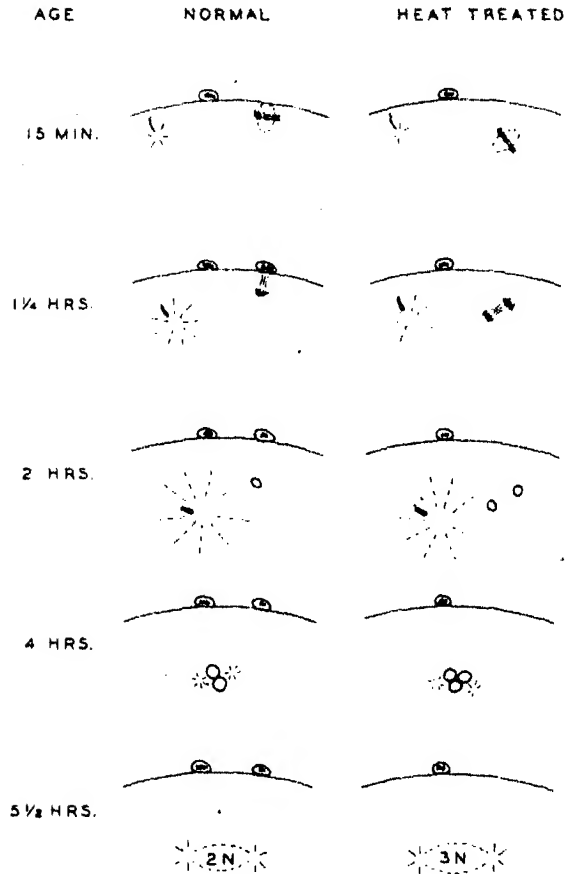


FIGURE 1

Diagrams of second maturation division and fertilization in normal eggs of *Triturus viridescens* and in eggs following exposure to 36°C. for ten minutes.

Only a small region of the egg near the animal pole is shown. Actually, the first cleavage spindle is located considerably deeper in the egg, near the center of the animal hemisphere.

Submergence of second maturation spindle in heat-treated eggs, formation of two egg nuclei, fusion of both egg nuclei with the sperm nucleus, first cleavage mitosis containing the triploid number of chromosomes (thirty-three).

For details see table 1.

inhibits primarily the shortening of the chromosomal spindle fibers that may be responsible for the early anaphase separation of the chromosomes. Whatever the cytological mechanism involved, direct observation of living eggs shows that the majority of heated eggs fail to give off the second polar body, and all embryos developing from such eggs are triploid.*

In view of the importance of temperature shocks in the production of polyploid animals and the complete absence of direct observations on the mechanism involved, a cytological study of the effects of short heat treatments on eggs of the newt, *Triturus viridescens*, was begun by the junior author in the winter 1946-1947. Eggs of this species were selected for this work because they were the object of the first experiments with cold and heat on amphibian eggs, and the cytology of fertilization is known in all details.²⁶ Fertilization in this species, as in the great majority of salamanders, is complicated by the occurrence of "physiological" polyspermy, which may be disregarded for the purposes of this article since it does not seem to be involved in the production of triploidy by abnormal temperatures. However, the presence of supernumerary sperm nuclei, which usually degenerate in the normal course of fertilization, offers a possible explanation of the origin of various exceptional chromosomal conditions, such as haploid-diploid or haploid-triploid mosaics.

Eggs were obtained during the winter and spring by subcutaneous implantation of one or two pituitaries from female leopard frogs. The newts were allowed to deposit the eggs on sprigs of *Elodea*, during which process they were fertilized in normal fashion by spermatozoa present in the sperm receptacle connecting with the cloaca. The experimental eggs were removed promptly, placed for ten minutes in an incubator at 36 to 36.5°C., and transferred to a constant temperature bath running at 19.6°C. Treated eggs and controls were fixed at intervals in Bouin's or in sublimate-acetic mixture, sectioned serially at fifteen to eighteen micra, and stained with either haemalum-fast green or Feulgen-fast green.

Thus far sections of thirty-six untreated eggs fixed up to 5½ hours from fertilization have been studied. Thirty-five show normal stages of fertilization corresponding to those described earlier;²⁶ one is abnormal. Among the forty-five heat-treated eggs that have been analyzed to date, twenty contain normal maturation and fertilization stages; presumably, these would have given rise to diploid embryos. Three eggs are definitely abnormal, probably unviable. The remaining twenty-two clearly show an inhibiting effect of the heat treatment on the second maturation division and illustrate the subsequent history of the mitotic mechanism in such affected eggs, as summarized in table 1 and figure 1.

It is clear from the evidence given that the most important effect of the heat treatment is not a general breakdown of the second maturation spindle but a submergence of the mitotic figure below the surface of the egg. In

TABLE 1
SECOND MATURATION DIVISION AND FERTILIZATION IN NORMAL AND HEAT-AFFECTED EGGS

APPROXIMATE AGE (AT 19.6°C.)	NORMAL EGGS (CONTROL AND EGGS NOT AFFECTED BY HEAT TREATMENT)	EGGS SHOWING HEAT EFFECT (SUBMERGENCE OF SECOND MATURATION DIVISION)*
15 mins.	Metaphase of second maturation division, spindle at right angles to and anchored at egg surface	Metaphase, spindle displaced below surface (1 egg)
30 to 40 mins.	Early to late anaphase, outer pole of spindle at surface or protruding slightly	Metaphase to late anaphase, spindle at varying distance below surface, perpendicular or oblique to surface (5 eggs)
1 hr.	Telophase, formation of second polar body in progress or complete, haploid set of egg chromosomes below surface	Late anaphase to early telophase, spindle at varying distance below surface, perpendicular or parallel to surface (2 eggs) [Abortive anaphase?, far below surface, two groups of chromosomes very close together, no spindle? (1 egg)]
1 $\frac{1}{4}$ to 1 $\frac{1}{2}$ hrs.	Telophase, second polar body given off, early stages in formation of egg nucleus	Telophase, spindle below surface (2 eggs) [Abortive late anaphase or early telophase far below surface, two groups of chromosomes very close together (1 egg)]
1 $\frac{3}{4}$ hrs.		[Single nucleus below surface, considerably larger than in controls at 2 hrs., restitution nucleus? (1 egg)]
2 hrs.	Small egg nucleus, still close to surface	Telophase, well below surface (1 egg); two small egg nuclei, identical in size and structure, close to surface, or at various distances below, not associated with any aster (4 eggs)
3 $\frac{1}{2}$ to 4 $\frac{1}{2}$ hrs.	Egg and sperm nucleus in contact, in various stages of early prophase, sperm aster divided	Three nuclei (two egg nuclei and one sperm nucleus) in contact, early prophase, sperm aster divided (3 eggs)
5 $\frac{1}{2}$ hrs.	Metaphase to anaphase of first cleavage mitosis	Late anaphase of first cleavage mitosis with 33 chromosomes at each pole (1 egg)

* Brackets mark three cases in which submergence of the spindle may be accompanied by failure of the spindle mechanism.

untreated eggs the spindle is perpendicular to the surface, and its outer pole is anchored in the cortex. During late anaphase, the outer pole begins to protrude slightly. At one hour, formation of the second polar body is in progress or completed. In heat-affected eggs, on the other hand, the spindle is displaced below the surface and usually rotated into an oblique

position. In the majority of cases, the separation of the two haploid sets of chromosomes at anaphase appears to be normal; and, during telophase, two small egg nuclei are formed. These are spherical and completely surrounded by yolk granules and thus easily distinguished from sperm nuclei in the same egg, since the latter are still elongate or club-shaped and always located near the center of a conspicuous sperm aster.

Three eggs, fixed between $3\frac{1}{2}$ and $4\frac{1}{2}$ hours from fertilization, show the crucial stage in the formation of the triploid chromosome complement, viz., the meeting of both egg nuclei with the principal sperm nucleus in the center of the original sperm aster. The three nuclei are associated with two small asters, resulting from the division of the sperm aster, which will occupy the poles of the first cleavage spindle as in normal diploid eggs. All nuclei are identical in appearance and show an early stage of prophase. There is no doubt that such a configuration will give rise to a normal, bipolar cleavage mitosis containing the triploid number of chromosomes. This was demonstrated directly by one egg fixed $5\frac{1}{2}$ hours from fertilization which showed a normal late anaphase of the first cleavage mitosis. Exactly thirty-three chromosomes, the triploid number, were countable at one of the two poles; however, the chromosome group at the other pole was too much disturbed by the sectioning process to allow an accurate count.

Three of the twenty-two heat-affected eggs (enclosed by brackets in table 1) indicate that the treatment, in addition to displacing the second maturation spindle from the surface, may also interfere occasionally with the separation of the chromosomes at anaphase through an effect on the spindle. In two eggs there is present an "abortive" anaphase, far below the surface, without a well-defined spindle and with the two groups of chromosomes practically in contact. On one of these eggs, the condition of the chromosomes suggests the beginning of telophase so that ultimately the formation of a single, diploid nucleus would seem to be unavoidable. In the third egg, a single egg nucleus is visible, which is considerably larger than the egg nucleus in untreated eggs, and possibly represents a diploid restitution nucleus resulting from an abortive anaphase. A single polar body is present in this series of sections; however, this cannot be taken as conclusive evidence of the suppression of the second maturation division since, in normal eggs, one or both polar bodies are frequently displaced or lost during embedding and sectioning.

A change in the position of the maturation spindle under the influence of abnormal temperature has been described previously by Gross⁵ in refrigerated eggs of *Artemia* in which the spindle may assume a position oblique or parallel to the surface. Submergence of one or both maturation divisions also occurs in unfertilized eggs of the worm *Urechis* following activation with either dilute sea water or ammoniacal sea water and,

probably, also after treatment with heat.^{26, 27, 28} A variety of cytological conditions have been described in such eggs which, normally, do not progress beyond prophase of the first maturation division without fertilization. If both maturation divisions are submerged, four haploid nuclei are present in the egg. If the first polar body is suppressed, one of the second maturation spindles may be located at the surface and produce a normal second polar body, leaving three nuclei, i.e., a triploid chromosome complement, within the egg. However, production of polyploid embryos from such eggs is prevented, as a rule, by the appearance of an aster with each egg nucleus, leading to multipolar mitosis and irregular distribution of chromosomes. In heat-treated, fertilized salamander eggs such irregularities of the first cleavage mitosis are avoided by the fact that the division center is brought in by the sperm, as in normal fertilization.

The cytological investigations on heated eggs of *Triturus viridescens* made so far show that the primary effect of the temperature shock is a severing of the outer pole of the second maturation spindle from its anchorage in the egg cortex and a submergence of the whole spindle below the surface. The polyploidizing effect of heat may thus be connected with a change in the properties of the surface film. A study of eggs fixed during the heat treatment may show whether there is also a temporary reversible effect on the spindle fibers themselves.

The observation that in some eggs submergence of the division figure is accompanied by a permanent modification of the spindle indicates a considerable variability in the response to heat treatment by the eggs of one species and suggests that eggs of other species may give a different cytological reaction. In *Rana pipiens*, for instance, inhibition of the spindle mechanism might well be the predominant feature either with or without submergence of the mitotic figure. Also, it should be pointed out that the cytological effects of prolonged refrigeration are not necessarily the same in all details as those of short heat treatments; although the ultimate consequences as regards the chromosome number of the embryos developing from such eggs are identical.

Summary.—Exposure of freshly fertilized eggs of the newt, *Triturus viridescens*, to 36°C. for ten minutes induces triploidy in over 50% of the surviving embryos. Cytological study of forty-five treated eggs fixed during the first five and one-half hours after treatment confirms the hypothesis that this is accomplished through suppression of the second polar body formation which, normally, is not completed until about one hour following fertilization. Twenty-two eggs give evidence of a submergence of the second maturation spindle below the egg surface. In the majority of these eggs, the separation of the chromosomes during anaphase and telophase proceeds normally, resulting in the formation of two haploid egg nuclei. Later on, both egg nuclei unite with the sperm nucleus while

the sperm aster divides in normal fashion to furnish the poles for the first cleavage spindle. During anaphase of the first cleavage mitosis, thirty-three chromosomes, the triploid number, may be counted at the poles of the division figure.

The principal effect of the heat treatment thus appears to be dislocation of the second maturation spindle from the egg surface, rather than an inhibition of the anaphase movements of the chromosomes leading to the formation of a diploid restitution nucleus, as had previously been postulated. However, observations of three of the twenty-two eggs indicate that failure of the spindle mechanism may be superimposed on the submergence of the mitotic figure.

* A reprint has just been received of a paper by Makino and Ozima (*Cytologia*, **13**, 55-60, 1943) in which they report the suppression of the second maturation division of fertilized eggs of the carp (*Cyprinus carpio*) following refrigeration. The division was arrested in anaphase and a diploid egg nucleus resulted.

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*ON THE RELATIONSHIPS BETWEEN THE FREQUENCY FUNCTIONS OF STELLAR VELOCITIES**

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1. In the study of stellar motions, methods of analysis generally follow a procedure in which the data of observation are tested for certain simplifying assumptions, i.e., one solves for a number of parameters associated with an assumed law of motion and proceeds to compare the motions given by observation with those predicted on the basis of the assumed law. While such a process is legitimate when examining the validity of the assumed law, it becomes inadequate whenever a further application of the law is required because it makes the data conform to an analytical expression at a stage in the analysis where the observations should retain their full unbiased influence.

A case in point is the statistical problem of determining the luminosity function for a selected group of stars in the neighborhood of the sun. Here the pertinent observations give us the distributions in proper motion and radial velocity for stars between certain limits of apparent magnitude. The problem is really one of evaluating the law of tangential velocities, since this law (when combined with the distribution in proper motion) will yield the distribution in parallax by a familiar theorem in stellar statistics. Now many investigators have in effect derived this law of tangential velocities on the basis of some analytical space velocity function known to approximate the space motions of the stars in question. Thus the extensive studies of van Rhijn¹ have relied rather heavily on the space velocity function first suggested by Schwarzschild. Strömberg in a series of papers² in effect assumes a quasi equivalence of radial and tangential velocity distributions, a procedure which is not justified, as has been pointed out by Oort.³ Again, the law of tangential velocities obtained by Luyten⁴ is based on a Maxwellian distribution of the peculiar motions.

In view of what has been said earlier it would be preferable to obtain

the distribution of the cross motions, without assuming a particular analytical form for the space velocity function, by establishing a numerical law of tangential velocities which will then serve the purpose of deriving the desired distribution in parallax. Several attempts have been made towards the solution of this problem. A notable contribution was made by Bok.⁵ Assuming the existence of a space velocity function in variant throughout the volume of space occupied by the stars under investigation, Bok considers the observed distribution in radial velocity along two axes normal to the direction of the region in which the law of tangential motion is required. By virtue of the assumed invariance of the space velocity function, these radial velocities are transposed to the region considered and there combined into a tangential velocity. The frequencies are computed by the rule of combining two independent events two at a time. The procedure presupposes that there is no correlation in radial velocity between both axes. On the basis of the ellipsoidal theory this assumption cannot be criticized since the theory is founded on the very hypothesis of independent distribution in space velocity components along three mutually perpendicular axes. However, a most general space velocity function of the type

$$\phi(u, v, w) \neq \phi_1(u)\phi_2(v)\phi_3(w)$$

clearly does not permit such an inference. The method is restricted by this consideration but it surpasses other methods in that it does not require a prior knowledge of solar motion, thereby bringing to zero the number of analytical parameters that enter the solution.

An important advance was made by Ambarzumian,⁶ who first showed that if we admit the existence of an arbitrary, invariant space velocity function, then the observed distribution of radial velocities for a group of stars lends itself to a determination of the frequency function of the space velocity components along a system of rectangular axes. Ambarzumian's analysis reduces to a minimum the assumptions regarding the space velocity function. His contribution appears to be of considerable significance although it seems to have been generally overlooked, perhaps in part because in certain cases the data on radial velocity are insufficient for a justifiable application of the method.

We shall show how Ambarzumian's attack can be directed towards a determination of the frequency function of the cosmic or space velocities themselves for stars of known velocity along the line of sight. On the basis of this result we shall then obtain an expression for the corresponding frequency function of the tangential velocities. Again we make the only assumption mentioned before, namely that the space velocity function is constant throughout the volume of space occupied by the stars to be analyzed.

2. In what follows we shall make use of a type of function not generally encountered in the literature. Let ρ denote the absolute value of the radial velocity not necessarily corrected for solar motion. We then define $g(\rho)$ as the average frequency distribution function of the absolute values of the radial velocities over the entire sky. It is to be understood that this function is obtained by averaging the frequency distributions tabulated for equal standard areas and that it is derived from the observations directly. Thus galactic concentration or any uneven surface distribution, if present, is removed in the process of evaluating $g(\rho)$. In particular the function g must satisfy

$$\int_0^\infty g(\rho) d\rho = 1.$$

In a similar way we define $F(V)$, where V is the linear space velocity, as the frequency distribution function of the linear space velocities over the entire sky. Of course these space velocities are to be considered as referred to whatever standard of rest is adopted for the radial velocities. Again we require

$$\int_0^\infty F(V) dV = 1.$$

Let $\vec{f}(V)$ denote the arbitrary (vectorial) space velocity function with respect to any set of rectangular axes. Isolate a single vector of magnitude V_i . This vector will generate over the surface of the celestial sphere a distribution in radial velocities $g_{\vec{V}_i}(\rho)$. Evidently the orientation of this vector is immaterial. Consequently any number of vectors of magnitude V_i may be conceived as evenly distributed over the surface of a sphere of radius V_i as far as their contribution to $g_{V_i}(\rho)$ is concerned. Thus for the purpose of evaluating $g(\rho)$ for all $V \geq \rho$, the space velocity function $\vec{f}(V)$ is equivalent to an isotropic density distribution

$$D(V) = \frac{F(V)}{4\pi V^2}$$

in velocity space.

We can now set up an equation relating $g(\rho)$ to $F(V)$ and we have (see Fig. 1)

$$g(\rho) d\rho = \frac{2}{4\pi} \iiint \frac{F(V)}{V^2} d\tau,$$

where $d\tau$ is the volume element in velocity space and the region of integration extends throughout the volume between two parallel planes normal to the direction of ρ (which is arbitrary) and separated by an amount $d\rho$. The factor 2 arises because we consider absolute values of the radial velocity.

Let $d\omega$ denote the surface element in the plane ABC . Then

$$g(\rho)d\rho = \frac{1}{2\pi}d\rho \int \int \frac{F(V)}{V^2} d\omega,$$

where the region of integration extends over the entire surface of the plane normal to ρ . Let (r, α) represent the polar coördinates of a point in this plane, measured with respect to any set of axes with origin at A . Then

$$\begin{aligned} g(\rho) &= \frac{1}{2\pi} \int_0^\infty \int_0^{2\pi} \frac{F(V)}{V^2} r dr d\alpha \\ &= \int_0^\infty \frac{F(V)}{V^2} r dr. \end{aligned}$$

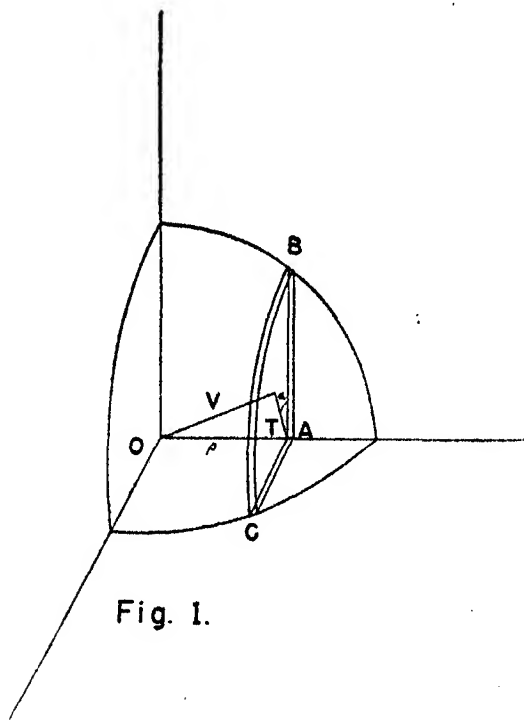


Fig. 1.

But $r^2 = V^2 - \rho^2$ and so, for given ρ

$$g(\rho) = \int_\rho^\infty \frac{F(V)}{V} dV. \quad (1)$$

We take the integral to be uniformly convergent by assuming $\lim_{V \rightarrow 0} \frac{F(V)}{V} = 0$.

The solution of (1) is then given by

$$F(V) = \left[-\rho \frac{d}{d\rho} g(\rho) \right]_{\rho=V}.$$

Everything on the right-hand side of this equation is known from observation and therefore the function F is determined.

3. Let $G(T)$ denote the average frequency distribution function of the tangential velocities over the entire sky. We require

$$\int_0^\infty G(T) dT = 1.$$

As before we may conceive of the space velocity function as an isotropic density distribution in velocity space. We now have

$$G(T) dT = \frac{2}{4\pi} \int \int \int \frac{F(V)}{V^2} d\tau,$$

where the region of integration extends throughout the volume between two cylindrical surfaces with axis along ρ and separated by an amount dT . The integration is again elementary and so we readily obtain

$$G(T) = T \int_T^\infty \frac{F(V)}{V\sqrt{V^2 - T^2}} dV. \quad (2)$$

In terms of the observed function $g(\rho)$ this result may be written

$$G(T) = -T \int_0^\infty \frac{g'(\sqrt{T^2 + \rho^2})}{\sqrt{T^2 + \rho^2}} d\rho. \quad (3)$$

4. Equations (1) and (3) express the solution of the problem.⁷ Inasmuch as these results are valid for a most general invariant space velocity function, we may treat as special cases those investigations of space and tangential motions based on a particular form of an invariant space velocity function.

Thus in the case of a Gaussian frequency distribution in radial velocities in any part of the sky, we have

$$g(\rho) = \frac{2}{\sigma\sqrt{2\pi}} e^{-\rho^2/2\sigma^2},$$

where σ is the dispersion in the space velocities. Here, by equation (1)

$$F(V) = \left[-\rho \frac{d}{d\rho} \frac{2}{\sigma\sqrt{2\pi}} e^{-\rho^2/2\sigma^2} \right]_{\rho=V} \\ = \frac{2V^2}{\sigma^3\sqrt{2\pi}} e^{-V^2/2\sigma^2}$$

in conformity with the law of chaotic velocities. The corresponding frequency distribution in tangential velocities is given by equation (2)

$$G(T) = \frac{2T}{\sigma^3\sqrt{2\pi}} \int_T^\infty \frac{V e^{-V^2/2\sigma^2}}{\sqrt{V^2 - T^2}} dV.$$

Putting $x^2 = V^2 - T^2$ this becomes

$$G(T) = \frac{2T}{\sigma^3\sqrt{2\pi}} e^{-T^2/2\sigma^2} \int_0^\infty e^{-x^2/2\sigma^2} dx$$

or

$$G(T) = \frac{T}{\sigma^2} e^{-T^2/2\sigma^2}.$$

A case of axial symmetry in the frequency distribution of space velocities is that of a single drift superposed on a Maxwellian distribution with respect to the local standard of rest. In the usual notation, we have

$$g(\rho) = \frac{1}{4\pi} \int_0^{2\pi} \int_0^\pi \frac{2}{\sigma\sqrt{2\pi}} e^{-(\rho - V_\odot \cos \lambda)^2/2\sigma^2} \sin \lambda \, d\lambda \, d\varphi,$$

where λ is the angular distance from the antapex and φ is the variable in longitude. Integration with respect to this latter variable is immediate and so

$$g(\rho) = \frac{1}{\sigma\sqrt{2\pi}} \int_0^\pi e^{-(\rho - V_\odot \cos \lambda)^2/2\sigma^2} \sin \lambda \, d\lambda.$$

The frequency distribution of the space velocities is given by

$$F(V) = \frac{V}{\sigma^3\sqrt{2\pi}} \int_0^\pi (V - V_\odot \cos \lambda) e^{-(V - V_\odot \cos \lambda)^2/2\sigma^2} \sin \lambda \, d\lambda.$$

Let $x = V - V_\odot \cos \lambda$ and the above expression becomes

$$F(V) = \frac{V}{\sigma^3 V_\odot \sqrt{2\pi}} \int_{V-V_\odot}^{V+V_\odot} x e^{-x^2/2\sigma^2} dx$$

or

$$F(V) = \frac{V}{\sigma V_{\odot} \sqrt{2\pi}} [e^{-(V - V_{\odot})^2/2\sigma^2} - e^{-(V + V_{\odot})^2/2\sigma^2}].$$

The corresponding frequency distribution in tangential velocities is given by

$$G(T) = \frac{T}{\sigma V_{\odot} \sqrt{2\pi}} \int_T^{\infty} \frac{e^{-(V - V_{\odot})^2/2\sigma^2} - e^{-(V + V_{\odot})^2/2\sigma^2}}{\sqrt{V^2 - T^2}} dV.$$

Let $x^2 = V^2 - T^2$ then

$$G(T) = \frac{T e^{-T^2/2\sigma^2}}{\sigma V_{\odot} \sqrt{2\pi}} \int_0^{\infty} e^{-(V_{\odot}^2 + x^2)/2\sigma^2} \left(\frac{e^{V_{\odot} \sqrt{x^2 + T^2} + T^2/\sigma^2} - e^{-V_{\odot} \sqrt{x^2 + T^2} + T^2/\sigma^2}}{\sqrt{x^2 + T^2}} \right) dx.$$

By expanding the exponential functions of terms involving V_{\odot} the above expression can be written

$$G(T) = \frac{2T e^{-T^2/2\sigma^2}}{\sigma V_{\odot} \sqrt{2\pi}} \int_0^{\infty} e^{-x^2/2\sigma^2} \left[\frac{V_{\odot}}{\sigma^2} + \frac{V_{\odot}^3}{3! \sigma^6} (x^2 + T^2) + \frac{V_{\odot}^5}{5! \sigma^{10}} (x^2 + T^2)^2 + \dots - \frac{V_{\odot}^3}{1! 2\sigma^4} + \frac{V_{\odot}^5}{2! 2^2 \sigma^6} - \frac{V_{\odot}^7}{1! 3! 2\sigma^8} (x^2 + T^2) + \dots \right] dx.$$

After term-by-term integration and some regrouping, we have

$$G(T) = \frac{T e^{-T^2/2\sigma^2}}{\sigma^2} \left[1 + \frac{V_{\odot}^2}{3! \sigma^2} \left(1 + \frac{T^2}{\sigma^2} \right) - \frac{V_{\odot}^2}{1! 2\sigma^2} + \frac{V_{\odot}^4}{5! \sigma^4} \left(1.3 + 2.1 \frac{T^2}{\sigma^2} + \frac{T^4}{\sigma^4} \right) - \frac{V_{\odot}^4}{3! 2\sigma^4} \left(1 - \frac{3! 2}{2! 2^2} + \frac{T^2}{\sigma^2} \right) + \dots \right].$$

This may be put in the form

$$G(T) = \frac{T e^{-T^2/2\sigma^2}}{\sigma^2} \sum_0^{\infty} A_j \left(\frac{T}{\sigma} \right)^{2j},$$

where

$$A_j = \frac{\left(\frac{V_{\odot}}{\sigma} \right)^{2j}}{(2j+1)!} \left[\frac{j+1}{1! (2j+3)} \left(\frac{V_{\odot}}{\sigma} \right)^2 + \frac{(j+1)(j+2)}{2! (2j+3)(2j+5)} \left(\frac{V_{\odot}}{\sigma} \right)^4 - \dots \right].$$

This result is identical with that obtained by Luyten.⁸

5. It can be shown that the assumed invariance of the space velocity function implies that $g(\rho)$ is a monotonically decreasing function. Hence, should equations (1) and (3) lead to negative or non-real frequencies for certain intervals in space velocity, it should be taken as an indication that within this interval the space velocity function is not invariant throughout the volume of space under investigation. In practice, a small local stream or "moving cluster," which contributes heavily to the distribution of radial velocities in a few areas of limited size, while being absent or relatively weak for the remainder of the sky, might produce peculiar distortions in the shape of $g(\rho)$. Obviously known members of local streams should be discarded in the analysis. The invariance of the frequency function of the space velocities is basic to our argument.

After our analysis had been completed, a recent paper by Colacevich⁹ was received. Formulae similar to those derived here are developed but the space velocity function is assumed to be spherically symmetrical. Colacevich's work may therefore be considered as a special case of our own investigation, since we did not find it necessary to make any assumptions beyond that of the invariance of the frequency function of the space velocities.

* The research has been carried out under a grant from the Office of Naval Research.

¹ *Groningen Publ.*, **34** (1923) and **38** (1925).

² *Mt. Wilson Contr.*, **395**, 93 (1930), *et seq.*

³ *Bull. Astr. Neth.*, **239**, 289 (1932).

⁴ These PROCEEDINGS, **11**, 87 (1925); Harvard Reprint No. 16.

⁵ *Harv. Circ.*, **400** (1935).

⁶ *Monthly Notices R.A.S.*, **96**, 172 (1936).

⁷ These relations can be deduced without recourse to geometry. Thus, if all stars form a single drift of velocity V_1 , we observe at an angle θ from the direction of the drift a radial velocity

$$\rho = V_1 \cos \theta \quad (1a)$$

and a tangential velocity

$$T = V_1 \sin \theta. \quad (1b)$$

Giving equal weight to equal solid angles (i.e., equal areas in the sky), the probability distribution of θ in the half-sphere in which $\cos \theta$ is positive is

$$dw = d \cos \theta = \sin \theta d\theta. \quad (2)$$

The probability distributions of ρ and T are then found by simple substitution of (1a) and (1b) in (2):

$$g(\rho)d\rho = dw = \frac{1}{V} d\rho \quad (3a)$$

$$G(T)dT = dw = \frac{T}{V\sqrt{V^2 - T^2}} dT. \quad (3b)$$

If now the distribution of space velocities over the entire sky is given by $F(V)$, we obtain by summing (3a) and (3b) over all possible V 's:

$$g(\rho) = \int_0^\infty \frac{F(V)}{V} dV$$

$$G(T) = T \int_0^\infty \frac{F(V)}{V\sqrt{V^2 - T^2}} dV.$$

* *Loc. cit.*

° *Publ. della Oss. Astr. di Arcetri*, Fasc. No. 64 (1948), p. 3

AMINO ACID CONSTITUENTS OF TISSUES AND ISOLATED CHROMOSOMES OF *DROSOPHILA*

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Introduction.—The chemical constitution of the chromosomes has been intensively studied ever since Miescher¹ discovered nucleic acid and extracted a protamine from salmon sperm. Pollister and Mirsky² characterize salmine (salmon sperm protamine) as lacking of tryptophane and tyrosine, and having a 31.6 per cent nitrogen content of which arginine makes up 89.2 per cent. Kossel³ prepared a protein which he called histone from nucleated erythrocytes. Histones are proteins distinguished for possessing basic properties, and according to Mirsky and Ris⁴ contain relatively little tryptophane. By enzymatic digestion and ultra-violet absorption measurements Caspersson⁵ established the presence of nucleic acid and protein in the salivary gland chromosomes of Diptera, whose significance was first demonstrated by Painter⁶ and Heitz and Bauer.⁷ Caspersson's findings were confirmed and extended by Mazia and Jaeger⁸ and Mazia,⁹ who considered the continuous protein framework of a chromosome to be composed of histone, but Kossel,³ the foremost investigator of histones, believed that only nuclei of certain kinds of tissues contained histones. *Chromosin* was extracted from various cells by Mirsky and Pollister.¹⁰ This substance can be divided into a soluble histone fraction, and a non-histone fraction which contains tryptophane. From lymphocytes of calf thymus, Mirsky and Ris⁴ isolated residual chromosomes, which were composed of arginine, cystine, tyrosine and tryptophane. In addition to the basic proteins, histone or protamine, Stedman and Stedman¹¹ isolated a second protein which was designated as *chromosomin*. Analysis

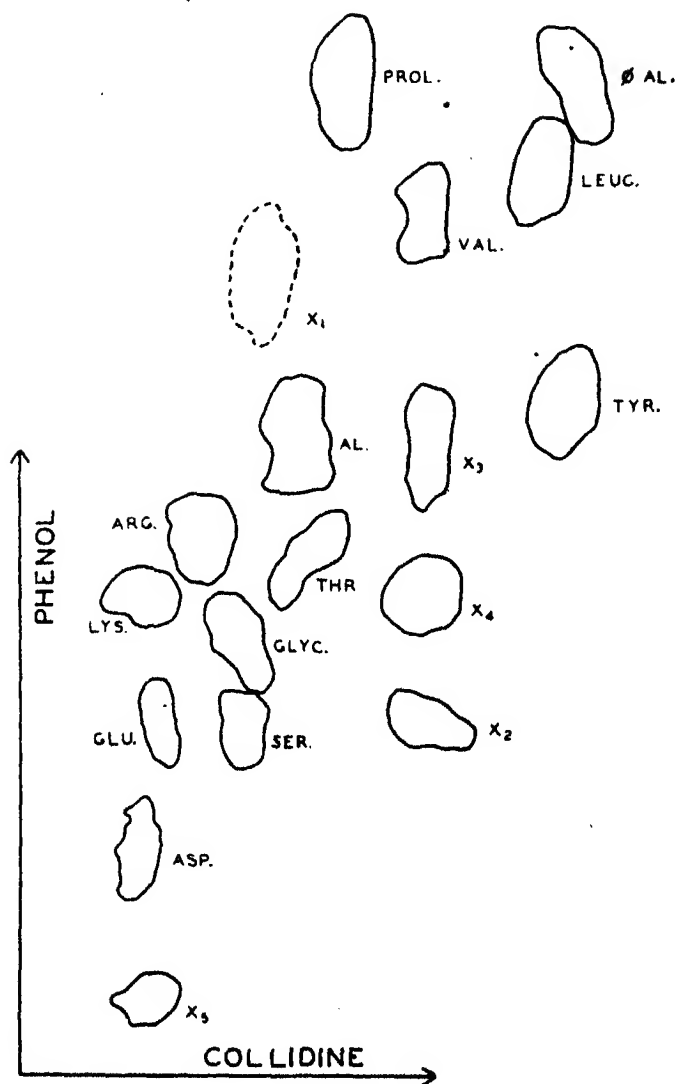


FIGURE 1

Tracing of a two-dimensional paper chromatogram of 36-hour hydrochloric acid digest of giant salivary gland chromosomes. X_1 , X_2 , X_3 , X_4 , and X_5 are as yet unidentified substances. X_1 is present in the brain tissue and is shown here to give its relative position on the chromatogram.

of chromosomin showed the presence of arginine, histidine, lysine, glutamic acid, tryptophane and cystine.

The similarity of viruses and genes may be of significance in view of the chemical and microbiological results obtained by Knight¹² on eight strains of tobacco mosaic virus. He suggests that in some cases in mutation among tobacco mosaic viruses there may be a stepwise change in amino acid content. Muller¹³ states that chemically the gene is of nucleoprotein nature and that viruses are composed of "*nothing but* nucleoproteins." As viruses fulfil the definition of a gene this lends support to the idea "that the most primitive forms of life consisted of nothing else than a gene."

With the introduction by Consden, Gordon and Martin¹⁴ of a comparatively simple technique for the detection of amino acids in very small amounts of material, the field of protein chemistry has taken on new aspects. One of the important biological applications of protein chemistry is in determining the exact nature of genetic material. This recent development of partition chromatography for identification of amino acids suggested this method of analysis. The Williams and Kirby¹⁵ modification of this technique has been used in these experiments.

Experimental.—Various kinds of tissues of *Drosophila virilis* were utilized for these experiments. The semen including sperm was obtained from several hundred adult males. Several tissues from mature larvae were also separated for analysis. These included brains, male gonads and salivary glands. In addition, chromosomes were mechanically isolated from salivary gland cells using a squash technique. This was done by staining in acetocarmine, breaking the cells and nuclear membrane by gently tapping a cover slip, washing the chromosomes in 45 per cent acetic acid, then picking up isolated chromosomes with a micropipette. As a control on the effect of staining, whole salivary glands were stained and tested. The tissues were hydrolyzed in 3 *N* hydrochloric acid for 36 hours at 100°C. After hydrolysis, the hydrochloric acid was removed by evaporation at a low temperature under reduced pressure. The residue was taken up in a small amount of water, 0.100 ml. for the sperm and chromosomes, and 0.200 ml. for the salivary glands, brains and gonads. An aliquot of this, 0.050 to 0.100 ml., was used to prepare two-dimensional chromatograms. Phenol was used as the first solvent followed by collidine.

Figure 1 is a tracing of a chromatogram showing the amino acids which were present in the salivary gland hydrolyzate. Several spots reacting to give a typical purple color with ninhydrin were observed which do not correspond to any of the amino acid known to occur naturally. Some of these have been observed by other workers, but differences in conditions during the preparation of the chromatograms are such that comparisons with earlier observations cannot easily be made.

Table 1 shows the amino acid composition of salivary glands, chromo-

somes, brains, male gonads and sperm. X_1 , which is present in the brain tissue, has been noted by us in a variety of other materials, and seems to be widely distributed. X_3 and X_5 have also been observed elsewhere. It is probable that some or all of these are amino acids yet to be identified.

The same amino acids were present in the stained as in the unstained salivary glands with the exception of X_4 . Its absence may be due to the action of the stain on this compound. Otherwise no difference could be detected between the stained and unstained glands on a qualitative nor quantitative scale. The sensitivity of the ninhydrin reaction is such that if arginine were present in the hydrolyzates it should have appeared on the chromatograms. On the other hand, amino acids such as histidine, tyrosine, phenylalanine and proline, may have been

TABLE 1

AMINO ACIDS	SALIVARY GLANDS UNTREATED	SALIVARY GLANDS STAINED	CHROMO- SOMES ISOLATED	BRAINS	MALE GONADS	SPERM AND SEMN
Aspartic	+	+	+	+	+	...
Glutamic	+	+	+	+	+	...
Serine	+	+	...	+	+	...
Glycine	+	+	+	...	+	+
Lysine	+	+	...	+	+	...
Threonine	+	+	...	+	+	...
Alanine	+	+	+	+	+	+
Arginine	+	+
Valine	+	+	+	+	+	+
Leucine	+	+	+	+	+	+
Tyrosine	+	+	...	+	+	...
Phenylalanine	+	+	...	+	+	...
Proline	+	+	+	+	+	...
X_1	+
X_2	+	+
X_3	+	+	...	+	+	...
X_4	+
X_5	+	+

present in the hydrolyzates and were not revealed in the chromatograms because of the low sensitivity of the reaction of these amino acids with ninhydrin. Cystine and tryptophane would be destroyed during acid hydrolysis.

Discussion.—Stanley¹⁰ in discussing viruses has pointed out that viruses in contrast to sperm nucleoproteins apparently do not have an excess of basic amino acids. Since certain properties of viruses have been compared with those of the gene it is therefore of interest to note that of the seven amino acids found in isolated chromosomes, valine, leucine, glutamic acid and glycine were present in all the virus strains tested by Knight.¹² Aspartic acid, alanine and proline were also present in all virus strains tested except the CV3 strain.

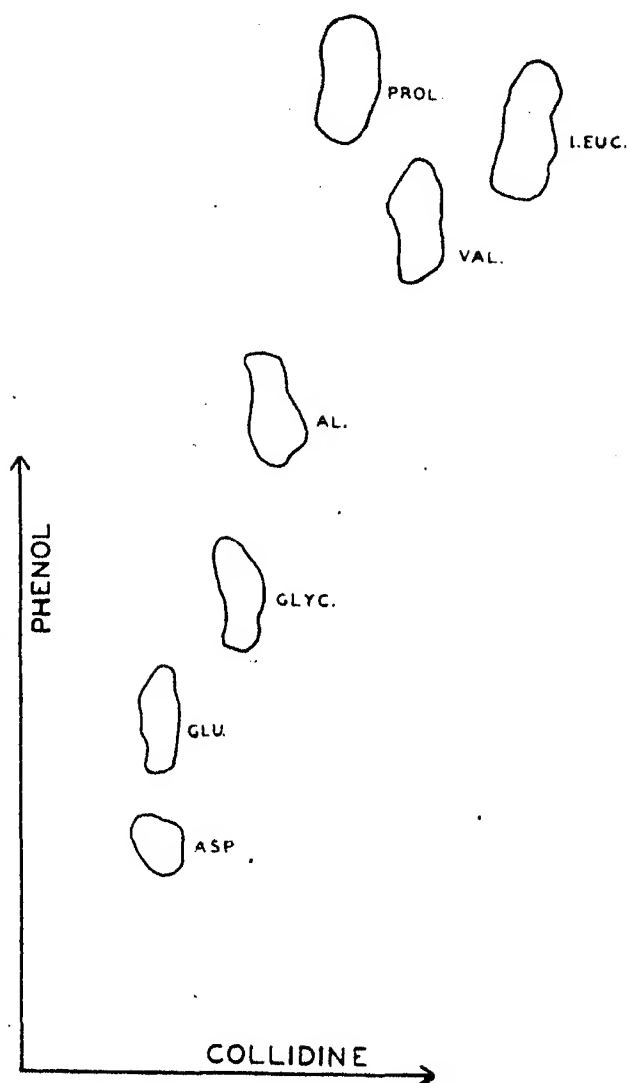


FIGURE 2

Tracing of a two-dimensional paper chromatogram of isolated chromosomes.

In this work aspartic acid, glutamic acid, glycine, alanine, valine, leucine and proline have been demonstrated in the hydrolyzate of chromosomes isolated from the giant salivary gland nuclei of *D. virilis* using the ascending paper chromatography microtechnique. The whole salivary gland contained these additional amino acids: serine, lysine, threonine, arginine,

tyrosine, phenylalanine and four unidentified compounds which give the ninhydrin reaction. Brains isolated from larvae contained the same amino acids as the salivary glands with the exception of glycine, arginine and three of the unidentified substances present in the salivary glands. Male gonads contained all of the amino acids present in the salivary glands with the exception of arginine and the unknown substances. Sperm and semen from adult males reacted to give glycine, alanine, leucine and valine. It should be noted that arginine is not present in these *Drosophila* testes, sperm and semen, or chromosomes, so it may not be an essential part of the protein material of sperm or chromosomes of this form.

¹ Miescher, F., *Die Histochemischen und Physiologischen Arbeiten*, F. C. W. Vogel, Leipzig, 1897.

² Pollister, A. W., and Mirsky, A. E., *J. Gen. Physiol.*, **30**, 101-116 (1946).

³ Kossel, A., *The Protamines and Histones*, Longmans, Green and Co., New York, 1928.

⁴ Mirsky, A. E., and Ris, H., *J. Gen. Physiol.*, **31**, 1-18 (1947).

⁵ Caspersson, T., *Skand. Arch. Physiol.*, **73**, 1-151 (1936).

⁶ Painter, T. S., *Science*, **78**, 585-586 (1933).

⁷ Heitz, E., and Bauer, H., *Zeit. Zellf.*, **17**, 67-82 (1933).

⁸ Mazia, D., and Jaeger, L., *Proc. Nat. Acad. Sci.*, **25**, 456-462 (1939).

⁹ Mazia, D., *Cold Spring Harbor Symp.*, **9**, 40-46 (1941).

¹⁰ Mirsky, A. E., and Pollister, A. W., *Jr. Gen. Physiol.*, **30**, 117-148 (1946).

¹¹ Stedman, E., and Stedman, E., *Nature*, **152**, 267-269 (1943).

¹² Knight, C. A., *J. Biol. Chem.*, **171**, 297-308 (1947b).

¹³ Muller, H. J., *Proc. Roy. Soc.*, **B134**, 1-37 (1947).

¹⁴ Consden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.*, **38**, 224-232 (1944).

¹⁵ Williams, R. J., and Kirby, H., *Science*, **107**, 481-483 (1948).

¹⁶ Stanley, W. M., *Currents in Biological Research*, Interscience Publishers, Inc., New York, 1946, pp. 13-24.

STUDIES ON THE BIOCHEMISTRY OF *TETRAHYMENA*. XIV. THE ACTIVITY OF NATURAL PURINES AND PYRIMIDINES*

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The requirement for purines and pyrimidines by *Tetrahymena geleii* was earlier demonstrated.¹ These requirements could be met by the addition of nucleic acid to the medium. At the time these studies were made it was necessary to add a crude extract from natural sources to supply the "Factor II" activity and this crude extract always contained some purine and

pyrimidine activity. Recently, however, it has been found that the Factor II requirement can be met by a highly active concentrate of a new vitamin-like substance, protogen² and increased pyridoxine, copper and iron,³ so that the crude concentrate originally employed can be omitted. With a medium entirely devoid of purines and pyrimidines it has been possible to reinvestigate their requirements in precise quantitative and qualitative terms, and to test the activity of numerous analogs as replacers, spacers and inhibitors. This communication deals with natural purines and pyrimidines⁴ in their effects upon the growth of the animal microorganism, *Tetrahymena*.

Material and Methods.—The organism used in these investigations was *T. geleii* strain W, grown in pure (bacteria-free) culture. The base medium

TABLE 1
BASE MEDIUM (ALL AMOUNTS GIVEN IN γ /ML. OF FINAL MEDIUM)

L-arginine HCl.....	83	Dextrose.....	2500
L-histidine HCl.....	36	Sodium acetate.....	1000
DL-isoleucine.....	113	Ca pantothenate.....	0.10
L-leucine.....	147	Nicotinamide.....	0.10
L-lysine HCl.....	116	Thiamine HCl.....	1.00
DL-methionine.....	94	Riboflavin.....	0.10
L-phenylalanine.....	70	Pteroylglutamic acid.....	0.01
DL-threonine.....	138	Biotin (free acid).....	0.0005
L-tryptophane.....	28	Choline Cl.....	1.00
DL-valine.....	76	Protogen*.....	0.375
DL-serine.....	157	MgSO ₄ ·7H ₂ O.....	100.00
L-glutamic acid.....	233	K ₂ HPO ₄	100.00
L-aspartic acid.....	61	CaCl ₂ ·2H ₂ O.....	50.00
Glycine.....	5	Fe(NH ₄) ₂ (SO ₄) ₂ ·6H ₂ O.....	25.00
DL-alanine.....	55	FeCl ₃ ·6H ₂ O.....	1.25
L-proline.....	175	CuCl ₂ ·2H ₂ O.....	5.00
L-hydroxyproline.....	75	MnCl ₂ ·4H ₂ O.....	0.05
L-tyrosine.....	67	ZnCl ₂	0.05
L-cysteine.....	3.5	Tween 85†.....	700.00

* Furnished by the Lederle Research Laboratories through the courtesy of Dr. E. L. R. Stokstad.

† Furnished by the Atlas Powder Company, Wilmington, Delaware.

employed is given in table 1. Growth responses were measured turbidimetrically as previously described.⁵ In all cases dose response experiments were conducted over wide ranges of concentrations of the material being tested and the experiments were repeated varying numbers of times. The results are all based on third serial transplants which, within any given experiment, were run in triplicate.

Results.—Dose response experiments were conducted to test the response of the organism to hydrolyzed yeast nucleic acid. Optimum growth was obtained upon the addition of 70 γ /ml. of final medium (Fig. 1). The ef-

fects of the various constituents of nucleic acid were then tested to determine the optimum levels of each free base and the nucleosides and nucleotides, in so far as they were available for testing.

Purines.—It was earlier shown¹ that guanine was by far the most active of the purines tested. The results we have obtained in this series of in-

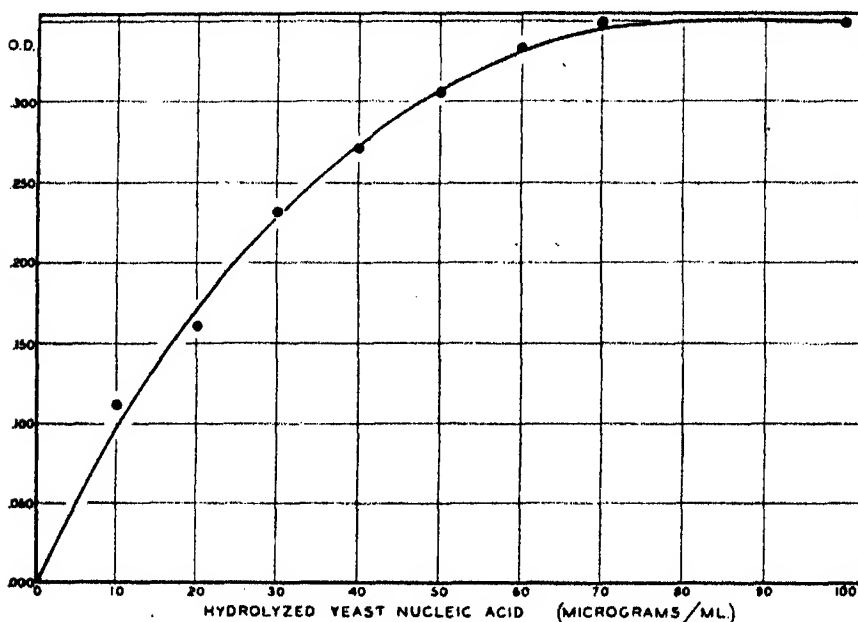


FIGURE 1

Dose response curve to alkaline hydrolyzed yeast nucleic acid.

TABLE 2

COMPARISON OF ACTIVITIES OF GUANINE, GUANOSINE AND GUANYLIC ACID. FIGURES REPRESENT THE AMOUNTS REQUIRED TO PRODUCE HALF-MAXIMUM GROWTH

	WEIGHT (γ /ML.)	MOLARITY (μ M)
Guanine	8.5	50.0
Guanosine	7.5	23.7
Guanylic acid	9.0	22.5
Guanylic acid + adenine (25 γ /ml.)	3.5	8.8
Guanylic acid + adenylic (25 γ /ml.)	3.5	8.8

vestigations indicate clearly that *Tetrahymena* is entirely incapable of guanine synthesis and this purine must be present in the diet in order that growth may occur. Moreover, contrary to our earlier conclusions which were based on a single concentration, the free base is approximately one-half as active as either guanosine or guanylic acid on a molar basis (table 2). Guanylic acid is slightly more active, on a molar basis, than the nucleoside

(Fig. 2). In accordance with these findings guanylic acid is the preferred source of guanine for work of this kind due to its greater solubility and also because maximum yields are consistently somewhat higher.

Adenylic acid supported slight growth when used with the crude Factor II preparation.¹ This may have been due to a slight guanine contamination in the sample of adenylic acid then used (adenine was without activity) together with the small amount of guanine in the Factor II preparation. In the present medium no growth results in the absence of guanine (or its nucleoside or nucleotide) over wide ranges of concentration of either ade-

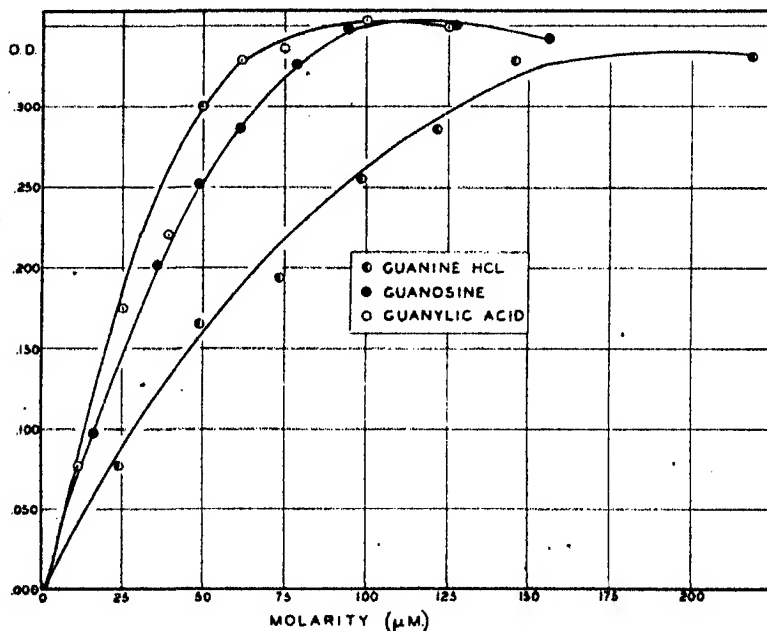


FIGURE 2

Dose response, calculated on a molar basis, to guanine, guanosine and guanylic acid. Base medium plus 50 γ /ml. of cytidylic acid.

nine or adenylic acid. Adenine can be synthesized from guanine but the reverse is not true for *Tetrahymena*. Both adenine and adenylic acid spare guanine, however, when added to the medium. Thus the addition of 25 γ /ml. of either adenine or adenylic acid reduces the requirement for guanylic acid for approximately half-maximum growth from 9 γ /ml. (22.5 μ M) to 3.5 γ /ml. (8.8 μ M) (table 2). Maximum yields are similar, however, whether adenine is synthesized by the organism or supplied in the medium (Fig. 3).

Xanthine, hypoxanthine and uric acid were tested for replacement and

sparing of guanine. Xanthine appeared to replace guanine but only at extremely high levels (1000 γ /ml.). Growth was very slow and the yields a fraction of maximum. We believe that there is every indication here of slight contamination with guanine of the xanthine used. When tested with suboptimal levels of guanylic acid (10 γ /ml.) there was some sparing action,

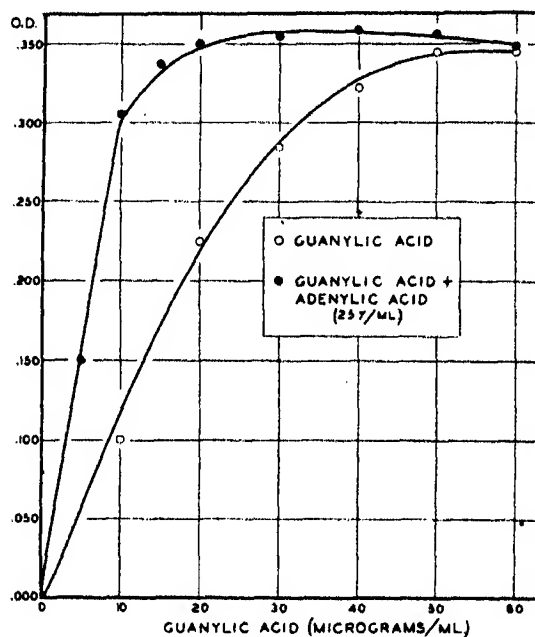


FIGURE 3

*Sparing action of adenylic acid. Base medium plus 50 γ /ml. of cytidylic acid.

TABLE 3

DOSE RESPONSE TO VARIOUS NATURAL PURINES IN THE PRESENCE OF SUBOPTIMAL AMOUNTS (10 γ /ML.) OF GUANYLIC ACID

AMOUNT ADDED (γ /ML.)	XANTHINE	OPTICAL DENSITY HYPOXANTHINE	URIC ACID
0	0.262	0.269	0.205
50	0.270	0.360	0.200
100	0.289	0.351	0.208
150	0.334	0.355	0.206
200	0.359	0.354	0.211
250	0.376	0.344	0.219

but again the high concentrations required would indicate a guanine contamination (table 3).† Neither hypoxanthine nor uric acid supported growth in the absence of guanine. Uric acid appears to be entirely inert and without either inhibitory or sparing action (table 3). Hypoxanthine, on the other hand, was active in sparing guanylic acid. The addition of 50 γ /ml.

of hypoxanthine together with suboptimal amounts (10 γ /ml.) of guanylic acid raised the growth to the normal maximum level. A comparison of the activities of hypoxanthine, xanthine and uric acid are shown in table 3.

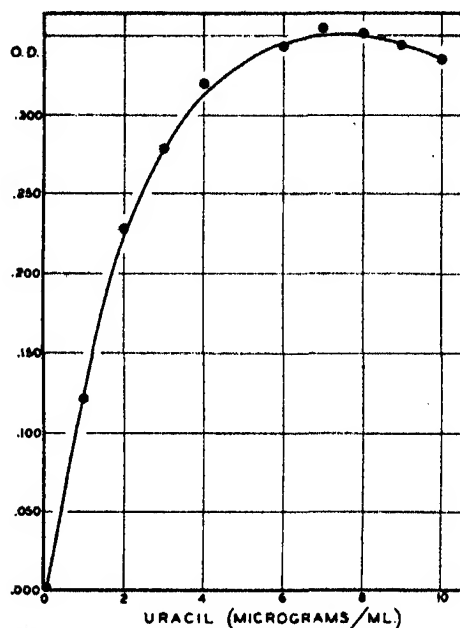


FIGURE 4

Dose response to uracil. Base medium plus 50 γ /ml. guanylic acid.

TABLE 4

DOSE RESPONSE TO THYMINE AND 2-METHYL-5-ETHOXYMETHYL-6-AMINO PYRIMIDINE (THIAMINE PYRIMIDINE) IN THE PRESENCE OF SUBOPTIMAL AMOUNTS OF CYTIDYLIC ACID (10 γ /ML.)

AMOUNT ADDED (γ /ML.)	OPTICAL DENSITY	
	THYMINE	THIAMINE PYRIMIDINE
0	0.220	0.231
100	0.225	0.229
200	0.252	0.206
300	0.264	0.199
400	0.271	0.189
500	0.280	0.184
600	0.301	0.175
700	0.293	0.163
800	0.268	0.154
900	0.265	0.149
1000	0.286	0.150

Pyrimidines.—No growth of *Tetrahymena* occurs when the medium is free of pyrimidines. The addition of either uracil or cytidylic acid produces

normal growth responses. Uracil is more active on a weight basis (1.5 γ /ml. for approximately half-maximum growth) than cytidylic acid (4.5 γ /ml. for half-maximum growth). On a molar basis, however, cytidylic acid is slightly more active than uracil (12.5 μ M and 13.4 μ M, respectively, for half-maximum growth). The addition of 7 γ /ml. of uracil (Fig. 4) or of 20 γ /ml. (Fig. 5) of cytidylic acid permits optimum growth. We have not had an opportunity to test the nucleotide of uracil in the present medium.

A most interesting fact brought out in this investigation was that cytosine is without activity for *Tetrahymena*. It will not serve as a replacement for cytidylic acid nor is there any sparing action. Apparently the organism

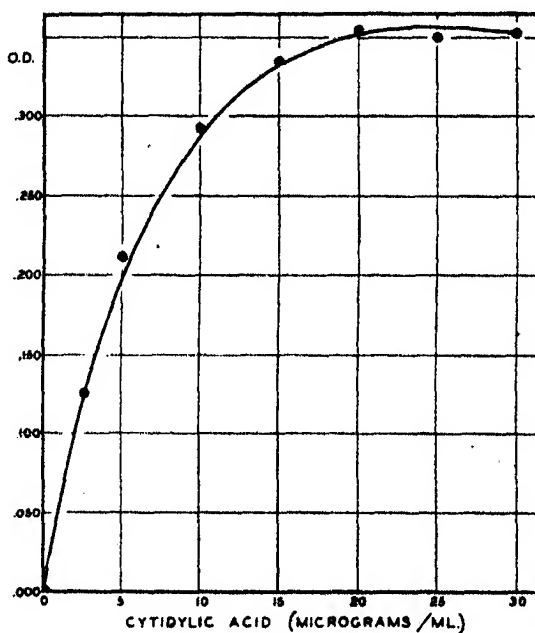


FIGURE 5

Dose response to cytidylic acid. Base medium plus 50 γ /ml. guanylic acid.

cannot metabolize this free base but can readily metabolize uracil. Similarly thymine is without activity for replacement and shows such weak sparing power as to indicate a slight chemical contamination of the sample tested (table 4).§

A test on the pyrimidine of thiamine (2-methyl-5-ethoxymethyl-6-amino pyrimidine) demonstrated that it is not able to replace uracil in the medium and becomes slightly inhibitory at high concentrations (table 4).

Discussion.—The failure of *Tetrahymena* to synthesize guanine from adenine and its undoubted ability to accomplish the reverse reaction contrasts this animal with higher forms in this one respect. Brown, *et al.*,⁶ showed

that when N^{15} labeled adenine was administered to rats the heavy nitrogen appeared in the guanine of the nucleic acids, while labeled guanine was not incorporated into the nucleic acids. It seems probable that of the enzyme systems for the interconversion of adenine and guanine, which were present in the animal prototypes, the enzyme catalyzing the synthesis of adenine was lost in the mammalian (and perhaps other) lines and that catalyzing the synthesis of guanine was lost in the ciliate line.

The nearly equal activity of the nucleoside and nucleotide indicates the relative difficulty of the riboside linkage as compared with phosphorylation.

As brought out earlier the evidence favors the view that this organism is incapable of utilizing xanthine, but hypoxanthine spares guanine. This may mean that hypoxanthine, with its unsubstituted 2-position, can be converted to adenine or it may mean that hypoxanthine has some other definite function in the organism (perhaps the synthesis of inosinic acid). No data from the present work are available on this point.

The similarities between our observations on the pyrimidine requirements of *Tetrahymena* and those of Loring and Pierce⁷ on *Neurospora* are striking. Loring and Pierce found that certain mutant strains would not grow in the absence of pyrimidines. Nucleosides and nucleotides of uracil were many times more active than the free base while cytosine was entirely inactive for one strain (1298) and nearly so for a second (H263). Cytidine and cytidylic acid were very active. We have not had an opportunity to test cytidine, uridine or uridylic acid⁸ but the results with cytosine as opposed to cytidylic acid are identical with the above on *Neurospora*.

It appears that *Tetrahymena* is able to accomplish the linkage between the base, uracil and the sugar to form the riboside but cannot do so when the base is cytosine or thymine. It may be that the enzyme catalyzing the production of riboside is so constituted that it will act only at the uracil surface and the amino group in the 6-position of cytosine or the methyl group in the 5-position of thymine block its function. It must be true, however, that thymine nucleotide and cytidylic acid are synthesized when the sole pyrimidine source is uracil, and that uracil and thymine nucleotides must be formed when the only source of pyrimidine is cytidylic acid. This would indicate that *Tetrahymena* possesses the necessary enzymes to methylate the 5-position without breaking the nucleotide down to the free base, and to substitute the amino for the keto group in the 6-position without breaking the pyrimidine-to-sugar linkage.

Summary.—The animal microorganism, *Tetrahymena*, requires an exogenous source of purine and pyrimidine for growth. None of the natural purines tested, except guanine, would support growth. Guanylic acid was slightly more active than guanosine and twice as active as guanine on a molar basis. Adenine, adenylic acid and hypoxanthine spare guanine, while uric acid is without activity. Xanthine possessed low sparing activity at

high concentrations but this can probably be attributed to a slight chemical contamination of the sample used.

Uracil or cytidylic acid can serve as the pyrimidine source for *Tetrahymena* while cytosine and thymine cannot. Cytidylic acid and uracil are nearly equal in activities on a molar basis. Neither cytosine nor thymine possesses sparing action. Thiamine pyrimidine (2-methyl, 5-ethoxymethyl, 6-amino pyrimidine) is slightly inhibitory at high levels.

* Supported by a grant from the U. S. Public Health Service and a grant recommended by the Committee on Growth acting for the American Cancer Society.

† Since this paper went to press a sample of synthetic xanthine was prepared for us by Dr. George H. Hitchings. This compound exhibited no guanine activity.

§ Since the above was written we have had the opportunity to test the following compounds: diammonium uridylate (prepared in the laboratory of H. S. Loring); cytidine (from the Levene collection); thymidine (sent to us by Dr. E. L. R. Stokstad). The uridylate and the cytosine exhibited the same activity, on a molar basis, as cytidylic acid. The thymidine was inactive. The range of concentrations of thymidine tested, however, was extremely limited due to the small amount of the compound available.

¹ Kidder, G. W., and Dewey, V. C., *Arch. Biochem.*, **8**, 293 (1945).

² Stokstad, E. L. R., Hoffman, C. E., Rezan, M. A., Fordham, D., and Jukes, T. H., *Ibid.* (in press).

³ Kidder, G. W., and Dewey, V. C., *Ibid.* (in press).

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⁵ Kidder, G. W., and Dewey, V. C., *PROC. NAT. ACAD. SCI.*, **34**, 81 (1948).

⁶ Brown, G. B., Roll, P. M., and Plentl, A. A., *Federation Proc.*, **6**, 517 (1947).

⁷ Loring, H. S., Pierce, J. G., *J. Biol. Chem.*, **153**, 61 (1944).

ON LEAF ARRANGEMENT IN *METASEQUOIA* *GLYPTOSTROBOIDES*

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Communicated by R. W. Chaney, October 1, 1948

Recently I had occasion to aid Dr. Ralph W. Chaney of this institution in arriving at a more exact understanding of the phyllotaxy of *Metasequoia glyptostroboides* Hu and Cheng, and the results of this investigation seem worthy of recording.

It has already been pointed out that the leaves of *M. glyptostroboides* are arranged decussately,¹ and indeed this is quite plain in some parts of the plant; however, modifications of this arrangement occur which make a more detailed statement of value.

The material examined includes a series of herbarium specimens from eastern Szechuan and northwestern Hupeh, China, collected by C. T. Hwa; seedlings grown by N. T. Mirov, of the California Forest and Range Experiment Station from seeds collected on the Hsieh expedition of 1947; and young trees and staminate spikes collected by R. W. Chaney in March, 1948.

The shoots of *M. glyptostroboides* are of two kinds, "long" shoots in which growth is usually indeterminate, and "short" shoots with usually determinate growth; the latter are deciduous as units. Long shoots can start growth as short ones, and vice versa. Conceivably, shoots of an intermediate nature may be found, although so far I have seen none.

The long shoots, as in other plants with shoot dimorphism, are found at the ends of the more rapidly growing branches and at the tip of the vertical axis. On the vertical axis there is little tendency for the leaves to become distichous by rearrangement, and it is here that the decussate phyllotaxy is most apparent (Fig. 1, $\times 1$). On the lateral branches, in young trees and presumably at the tops of older ones, the decussate arrangement may still be obvious near the vertical axis where the square stem of the lateral branch is oriented horizontally and vertically, as seen in cross-section. But a few inches from the vertical axis the stem gradually twists to assume a position of about 45° from the horizontal, and the leaf pairs bend on their bases toward the horizontal, thus more or less approaching a two-ranked condition. Here there may be found some twisting of the stem between nodes such as will be described under the short shoot, but only a very slight twist has been observed.

The short shoots are borne in the axils of the leaves of the long shoots (Fig. 2, $\times 1\frac{1}{2}$), and also in leaf axils of short shoots in many cases. They usually come to lie more or less parallel with the ground, as do the mature leaves of the shoot. Frequently short shoots will continue growth, branch, and may start growth as long shoots. Branching short shoots apparently may be the only means of growth in some of the slower growing branches; further examination of specimens taken during the growing season from mature trees is necessary to ascertain this point satisfactorily. Most often the leaves of short shoots are much more closely spaced than those of the long, but sometimes a short shoot will grow rapidly with widely spaced leaves. In such cases the most reliable means of distinguishing between the two shoots is by comparing their growing terminal buds, which, with their subtending leaves, have quite characteristic forms. Also, those of the long shoots (Fig. 1) often have a darker, more blue-green color than those of the short shoots (Figs. 3 and 4, $\times 1$).

Leaves of the short shoots are also decussately arranged, but it is only in the terminal bud that this arrangement is unmodified. Figure 5 ($\times 35$) is a camera lucida drawing of a cross-section of such a terminal bud, made at the

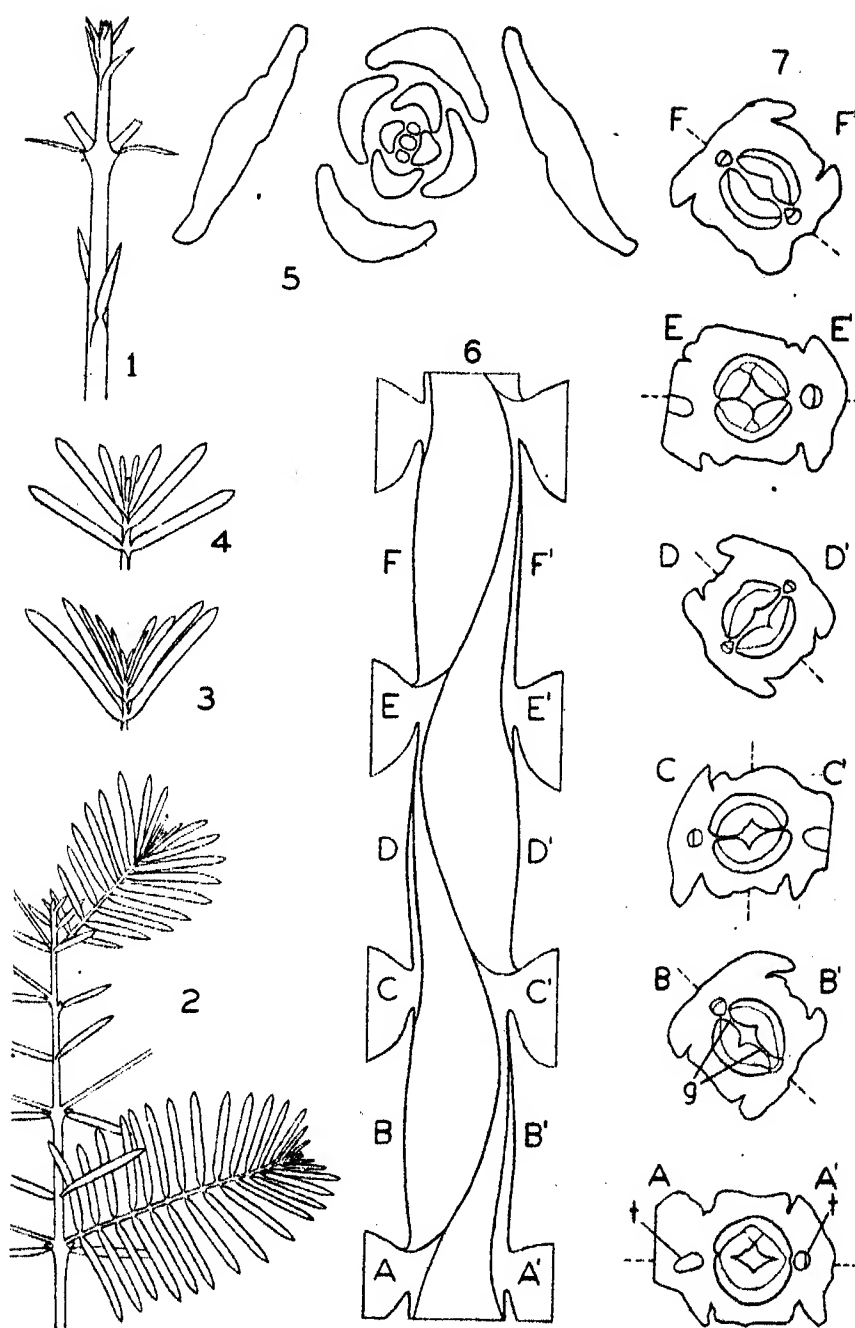


FIGURE 1

level of the apical meristem. Here are seen six pairs of leaf primordia, five of them in regular decussate arrangement. However, as each successive leaf pair enlarges and opens outward from the bud, its node twists so as to bring the leaf bases into a plane approximately parallel with the ground. "Node" is here used to indicate only the attachment point of the leaf blade, disregarding the decurrent base. The change from non-twisted to twisted internodes is usually quite abrupt: there is no twist between the nodes of the fifth and sixth primordia shown here, and there is a 75° twist between nodes of leaves six and seven. The turning of the leaf pairs is evidently dependent on their attaining a certain size, when the force produced by the acting stimulus is sufficient to bring about a turning. The nature of the stimulus is not certain; it seems likely that light is the primary factor. Application of the force is apparently at the nodes, as would be expected. Alternating nodes rotate respectively clockwise and counterclockwise, bringing all the leaf bases into the same plane with an angular twist of slightly less than 90° between any two nodes.

When the bud is growing vigorously (Fig. 3) one pair of enlarging leaves closely follows another, and as one starts to turn toward the plane of the mature leaves in one direction, the next is apparently already exerting some force in the opposite direction; this results in the terminal bud remaining oriented at about 45° from the leaf plane, as seen in end view. However, when growth has slowed down considerably (Fig. 4), one pair of leaves may enlarge and turn before the next has reached an appreciable size, and in this event the whole bud will turn as the leaf pair turns, there being no opposing force. When this happens the next oldest leaf pair is automatically brought at right angles to the leaf plane, and when it in turn opens outward, its node will rotate in the opposite direction, bringing the following leaf pair to a right angle position, and so on. In either case the effect on the stem is the same.

In order to illustrate the twisted nature of the stem, figure 6 has been drawn to show diagrammatically an enlarged segment of the mature stem of a short shoot, and in figure 7 ($\times 21$) are shown camera lucida drawings of stem sections (material from C. T. Hwa, collection no. 179) taken from positions corresponding to those indicated in figure 6. The sections are shown as seen from the tip of the shoot looking toward the base and are taken in that order; the dotted line indicates the level of the stem as seen in *A-A'*.

In section *A-A'* the two leaf traces (*l*) are seen as they have just entered the cortex, and the shape of the pith indicates that the leaf gaps (*g*) extend as grooves in the xylem above the points of actual junction of the traces. In *B-B'* the position of the leaf traces and gaps indicates that the whole stem is twisted at a 45° angle. One leaf trace has already joined the vascular cylinder, and the other is about to join it. The rather frequent

occurrence of leaves which are not quite opposite one another at the node is probably of little significance; in some such cases the leaf traces join the vascular cylinder unequally and in others they do not. Section *C-C'* completes the 90° twist; the traces of *A-A'* are fully fused with the vascular cylinder and those of *C-C'* have entered the cortex. Again the leaf gaps of the traces of *A-A'* are seen to extend as grooves, this time below the fusion points of the traces. The groove extensions of the leaf gaps give anatomical evidence of the decussate phyllotaxy, since where grooves of adjacent nodes overlap, those of one node are always 90° apart from those of the next. *D-D'* and *E-E'* show the same procedure occurring, this time in the reverse direction, and therefore there is no continuous spiral. *F-F'* illustrates the twist starting again clockwise as in *B-B'*.

The amount of twist between nodes may vary greatly from one shoot to another; in one very young tree the variation was from almost no twist in some to a full 90° twist in others. Even in the same shoot the amount of twist varies considerably, apparently at random. In one observed case circumstances had produced a 100° twist between two nodes. The average seems to be roughly 75°. Differences in amount of twist are compensated for by a bending of each leaf on its base toward the horizontal, so that all short shoots appear flat-ranked. However, in the older short shoots which have continued growth and increased considerably in diameter, there is frequently a reorientation of the leaves on their bases away from the horizontal plane, so that the position of the leaf indicates more nearly the position of the leaf base. This gives the appearance of the stem's untwisting, but it is very doubtful if such a thing occurs.

The twisted stem so typical of the short shoot is also found in the majority of cases in the spike bearing the staminate strobili. Again there is a great deal of variation in the amount of twist between nodes, and some spikes show almost none. Investigation shows the internal situation to be much the same as in the short shoot, the traces from the leaves subtending the strobili and the leaf gaps following the same pattern.

The shoots bearing the ovulate strobili in most instances show little or no twisting between nodes. Details have not been investigated but it seems reasonable to assume that where twisting is present the behavior of traces and gaps will be essentially the same as in the short shoot.

No attempt has been made here to compare *M. glyptostroboides* with other apparently related genera, such an undertaking being beyond the scope of this paper. It is hoped that these facts may help in leading to a better understanding of the nature and relationships of this interesting tree.

¹ Stebbins, G. L., Jr., *Science*, 108 (2796), 95-98 (1948); Hu, H.-H., and Cheng, W.-C., *Bull. Fan Mem. Inst. Biol.*, N. Ser., 1 (2), 153-161 (1948).

AN ASCENT OF KOROYANITU

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Koroyanitu is the high point of the Mt. Evans Range, a high and narrow ridge rising from the grassland hills of northwestern Viti Levu. This largest island of the Fijian archipelago is physiographically separable into two parts by a central mountain axis dominated by the Rairaimatuku Plateau, an extensive forested area with an average elevation of perhaps 800-900 meters. The western half of Viti Levu and a strip along the north coast are relatively dry savanna and bush, locally known as the *talasinga* country. The eastern half is wet forested land, called by the natives the *veikuu*. The line of demarcation between these two types of land is often well defined. The Mt. Evans Range lies entirely within the dry zone of Viti Levu, but in height it is third on the island, exceeded only by Tomanivi, near the northern edge of the Rairaimatuku Plateau, and Korombasambasanga, south of the plateau. Because of its altitude (1195 meters), the Mt. Evans Range receives a heavy rainfall on its upper slopes and is well forested above an approximate 700 meters. Isolated from the main forest of Viti Levu by many miles of low, broken, reed-covered *talasinga*, the forested upper slopes of the Mt. Evans Range offer a tempting goal to a botanist. This range was selected as my first objective during a recent botanical reconnaissance of Fiji in 1947-1948.¹

The Mt. Evans Range had the further advantage, in the eyes of a botanical explorer, of being essentially unknown botanically,² although it had been explored by some of the surveyors who in recent years have provided the Lands and Survey Department, of Suva, with excellent detailed maps of the larger islands.

As headquarters for my five-week reconnaissance of the Mt. Evans Range, the Fijian village of Nalotawa was selected. Nalotawa lies at the eastern base of the Mt. Evans Range and has an elevation of about 550 meters, being situated in a wooded valley in the grassland, but only a mile or two from the beginning of the forest on the slopes of the range. On a prearranged day about 15 Fijian "boys" from Nalotawa met me and my "head assistant," a capable young Fijian named Tanieli Rawangga, at a sugarcane plantation in the valley of the Mba River, to which point we had brought our supplies by truck. The professional tropical plant collector cannot travel light; he must have a supply of collecting paper, blotting-paper driers, corrugated boards, presses, straps, stoves and kerosene for drying specimens, axes, machetes, sundry other tools, and staple food supplies for whatever period he plans to remain in "the bush." My 15 carriers

were loaded with 30 or 40 pounds each when we finally left the road. In making contact with village people and in arranging for carriers it is quite essential, in a country like Fiji, to work through the chiefs and to have the proper official introductions. Throughout my work in that Crown Colony I have enjoyed the kindest coöperation from British officials and from Fijians of all classes, from "Rokos" and "Mbulis" (the principal provincial and district chiefs) to the simplest villagers.

Our route led westward from the Mba River over gently rolling grassy hills for a few miles, and then upward with increasing precipitousness along the sharp lower spurs of the Mt. Evans Range. These sharply dissected lower slopes are covered with mixed grasses; in northwestern Viti Levu an introduced grass, *Pennisetum polystachyon*, has taken control of vast areas and has swamped the native vegetation. It is a tall grass, forming dense stands two meters or more in height, and its leaves and slender inflorescences encroach upon the trail, giving one the impression that one is struggling through an endless wheat field, but a wheat field as nearly vertical as horizontal. The trail follows the crests of ever-steepening spurs, from which one looks down startling slopes to the little bush-filled valleys far below. After about ten miles of arduous walking, we came abruptly to the crest of a ridge and saw below us, among mango and orange trees, the houses of Nalotawa.

The remaining hours of our first day were spent in a continuous pow-wow with all the adult male members of the community, in the large and excellently built native house of Tui Yakete, the ranking chief of a group of five adjacent villages. From time to time we were served *yanggona* in the ceremonial Fijian manner; this beverage, the *kava* of the Polynesians, is prepared from the root of *Piper methysticum* and is consumed on every plausible occasion by the Fijians. No business transaction can be carried on, no arrival heralded, no departure consummated, without recourse to the *yanggona* bowl and its attendant rigid ceremony. Coming at length to the point, Tanieli explained my work to the villagers and presented my official credentials; Tui Yakete reciprocated by turning over to us his house as living and working quarters, assigning men to work with us in the field, and promising to supply us with staple native foods. Finally I was able to seek my sleeping mat, but this evening—and most evenings, as I learned—many villagers stayed in the house after I retired and continued their conversation quite uninhibited, from time to time dozing on the mats. It is considered impolite in Fiji to terminate a visit abruptly.

For the first few days I collected on the slopes of the range, following the same route higher each time. The route selected for our ascent of the range lay over the saddle of a massive eastern outlier, which itself is dominated by a huge pinnacle of bare rock known as Nairoso or "The Thumb." From the base of the actual pinnacle, which has an elevation of more than 1000 meters, we were able to obtain excellent views over the countryside and to plan

our further route. This followed a narrow ridge to the eastern flank of the main range itself. Several of my willing assistants spent a day cutting a rough track through the dense vegetation of this ridge. Directly below the main peak of Koroyanitu they located a fairly level spot on the ridge, not far from a tiny stream, cleared a small camp-site and set up a canvas fly.

I usually planned to have four or five Fijians accompany me on field trips, as this many helpers are desirable in a forested country where cutting of tree specimens is a frequent necessity; tropical woody specimens are so bulky that, if several duplicate sets are obtained, a quantity of material needing several men to carry is the result of a good day's work. When the day arrived for our ascent of Koroyanitu, however, ten boys of the village presented themselves. Although the peak is not distant from the village, only one of these boys had previously ascended it, and he had been a surveyor's carrier many years before. With this considerable retinue of ten local boys and Tanieli, carrying collecting supplies and food for a couple of days, I left Nalotawa on May 1 to put "operation Koroyanitu" into effect. We were accompanied by numerous hunting dogs, in case we should locate a wild pig. On an earlier day this hunting pack had had a brush with a wild pig with rather unsatisfactory results from a canine point of view, one dog being completely eliminated and another being lost for three days in the *veikau*, finally finding his way home badly crippled. One has to admire the temerity of these Fijian hunters, who track down and kill often dangerous wild pigs with no other weapon but a metal pike.

We entered the forest on the slope of Nairosa and proceeded along the now familiar lower part of the trail. It was a fine day, but even so the forest was dark and gloomy, as the sun does not penetrate to the ground through the dense canopy of the trees. Underfoot were boulders of all shapes and sizes, and the wet clayey soil, so characteristic of the Fijian hills, made the walking difficult as the slope gradually steepened. Soon the spectacular peak of Nairosa towered above us, discernible through occasional breaks in the foliage. Passing through the saddle below this pinnacle, we emerged on the long curving ridge and followed it westward. Our route was now more level but even more difficult underfoot, as the vegetation of the exposed ridge was windswept and tangled, interlaced with scrambling ferns and the branches of a sprawling *Freycinetia*.

Soon after noon we had crossed the diminutive stream which represents the "highest water" on this slope, and ten minutes' climb above this I found the camp-site which my advance party had selected; indeed they had located the only even approximately level spot in the vicinity. Here we had lunch and rested. After a while I set some of the boys to work extending our minute clearing, others to cutting a trail toward the summit, and took a couple with me to collect in the immediate vicinity. From our camp-site we could not see Koroyanitu, but a frontal and slightly lower peak, Koro-

ynasamama, towered above us; it is Fijian custom to give each peak a separate name, but actually I could not ascertain that the natives had any name for the entire range which Europeans in the vicinity call the Mt. Evans Range. The vegetation near our camp was of upland crest type, with gnarled and dwarfed trees jammed together in a dense moss-cloaked forest. Few individual trees in such a habitat exceed six or eight meters in height; only on the sheltered slopes of ridges, or in deep wet valleys, do individual trees attain a larger size. The components of this upland forest are exceedingly varied and so mixed that no species can be considered dominant. Of frequent occurrence among the trees, however, were two conifers, probably *Podocarpus* spp.,³ a small-leaved *Alphitonia*, and species of such genera as *Alstonia*, *Hedycarya*, *Tapeinosperma*, *Aglaia*, *Elaeocarpus*, *Dolicholobium*, etc. Several undershrubs of the beautiful genus *Cyrtandra* bore attractive white flowers, as did various species of the ubiquitous genus *Psychotria*. The whole mass of woody vegetation, at this altitude, is interlaced with lianas of the genera *Medinilla*, *Maesa*, *Agatea*, and *Morinda*, and quantities of epiphytic orchids, ferns and small cryptogams cover the wet branches and the vegetation-debris underfoot. Several hours of diligent collecting gave us a fair sample of the vegetation surrounding our camp, and at the end of that time we were all ready to enjoy our supper of yams, *ndalo* (*Colocasia*), tinned meat and tea. The members of my crew left at the camp-site had occupied themselves to good purpose, making rough shelters for themselves and even clearing a "line" through the tangled forest to give us a perfect view of the pinnacle of Nairoso, with which we were now on a level. This concession to scenic appreciation I particularly approved at dawn the next morning, when the rising sun outlined the black pinnacle against a red sky, setting up for me a kodachrome picture which serves to remind me that on rare occasions, at least, it can be a pleasure to arise at dawn.

We were indeed fortunate to have a second clear morning, for in the Fijian mountains one cannot expect two consecutive days without rain. Even on clear days the clouds gather quickly on the summits, and so one's only hope of good visibility from the peaks is to reach them early in the day. Starting from our camp-site immediately after a quick breakfast of yams, *ndalo*, and tea, we followed the trail which our advance guard had slashed the preceding afternoon. This final ascent was a bitterly steep and rough climb among roots and huge boulders, winding around the base of Koroynasamama, and finally up a precipitous cliff, to emerge suddenly from the impeding tangle of vegetation onto the summit ridge. Although it is difficult, from below, to pick out the high point of the Mt. Evans Range, one has no doubts when standing on Koroyanitu, for this knife-edged crest emerges from the jumble of ridges and clearly overtops its neighboring peaks. We took turns standing upon the high point, which is a conglomerate boulder about two meters in diameter. The view from this vantage point, on our

remarkably clear morning, was spectacular. To the west we looked down on the Viti Levu coast and the town of Lautoka, while the entire Yasawa chain of islands was spread before us, lying hazily on the blue sea, dwarfed by distance and made insignificant by our height. To the south the mass of the Mt. Evans Range impeded much of the view, but the distant Koromba,⁴ green with its summit forest, stood above the yellowed and sharply eroded grassland hills. To the east lies Nairoso, now well below us, and the mass of Viti Levu, its rough undecipherable topography leveling off in the distant Rairaimatuku Plateau; on this day even Tomanivi was briefly visible, but soon it was engulfed by the cloud mass which so often lies upon the central plateau. By ten o'clock the visibility had dwindled to the immediate foreground, and the rest of the day was intermittently foggy and cloudy.

The summit ridge of Koroyanitu proved to be a collector's dream, and for a long time I could only put into the press as rapidly as possible the offerings brought by my interested and observant boys, who now knew that I wanted ten duplicates of each flowering or fruiting plant. Eventually we worked southward on the ridge for a mile or so, collecting carefully as far down the steep slopes as was consistent with safety. At noon we made a clearing from which we could observe the nearby summit of Mbotilamu, and here we had our lunch of yams, *ndalo*, tinned fish, and tea. The vegetation of the summit was superficially similar to that of the camp-site, but many different species were observed and collected. Noteworthy were four distinct species of *Piper* (the genus which provides *yanggona*), and on the highest boulder I was pleased to observe a plant of *Medinilla longicymosa*, the beautiful pink-bracted melastome which occurs only on Viti Levu, and there only on a few of the higher peaks. A locally common fern is *Oleandra Parksii*, most unfernlike in appearance, with its erect rhizomes forming stiff thickets a meter high and its fronds simulating the leaves of an angiosperm.

When we had returned to the camp-site it was three o'clock, and a storm was rapidly gathering. As I contemplated the prospect of spending a wet night on this exposed spot and the difficulties of returning down a wet trail, I suggested to the boys that we break camp and make a quick return to the village before dark, if possible. This necessitated a conference of all hands, in which the pros and cons were carefully discussed, regardless of the moments slipping by. The ayes won out, and so the camp was folded up and the remaining yams and *ndalo*, which had thoughtfully been cooked at breakfast time, were eaten (for it would have been foolish to carry them back to the village, and improvident to abandon them). At length we departed, slithering down the wretched path, among the gloomy trees, around the dark boulders and massive roots, at a definitely good pace. The decision was a fortunate one, for hardly had we reached the edge of the forest before we were caught in the advancing storm. The last part of the descent, in semidarkness over the rain-soaked grassy trail, brought us to Nalotawa

before total darkness. Clouds enveloped the mountain throughout the night and the next day, and we had no cause to regret our precipitate retreat. Mountain camps in the rain are scarcely enjoyable, and one develops an appreciation of the comparative comforts of native villages.

After such a profitable trip as that described above, I had to spend two or three long days working in the village, making notes and preparing, pressing and drying the specimens. Such days were pleasantly spent, as a good Fijian house makes a fine headquarters, and one is assured of congenial company. When I had finished my work in the evening, many of the villagers would come to visit and we would sit on the mats drinking *yanggona* and conversing, by way of Tanieli as my interpreter. Fijians are tireless conversationalists, and one hears, every evening, a steady hum ascending from the village houses. But they are definitely unacquainted with the spirit of Shakespeare's lines which Proust uses to introduce his great novel:

"When to the sessions of sweet silent thought
I summon up remembrance of things past, . . ."

The foregoing episode, covering a few days in the life of a plant taxonomist, may serve to acquaint the reader with the first step in a phytogeographical problem. In most tropical countries we are still in the "alpha" phase of plant classification, when the flora is so imperfectly known that novelties may be expected and gaps in the distributional data are obvious. During the nine months which I spent in Fiji in 1947-1948, some 26,000 herbarium specimens were collected and prepared, these representing upward of 2900 field numbers. The first set of material has been deposited in the herbarium of the Arnold Arboretum, and the nine duplicate sets will be sent to some of the other large world herbaria. Supplementary material consisting of 226 wood samples and flowers and fruits in preservative was also acquired.

What is the purpose of such collections? The unattainable goal of systematic botanists is to record the distribution of all plants and to classify them in a system which will, in some manner, depict their phylogenetic history. The primary purpose of our great herbaria is thus to present a picture, by means of representative specimens, of the composition of the modern plant world. These herbaria must be the chief basis for future monographic and phytogeographical studies. But they also serve many others beside the taxonomist; the economic botanist, the ethnobotanist, the morphologist, the geneticist, and students of many other disciplines seek much of their basic data in the collections and publications of systematic botanists.

¹ The mentioned botanical excursion, of which this paper briefly details a single episode, was made by the writer under the auspices of the Arnold Arboretum of Harvard University and was largely financed by a John Simon Guggenheim Memorial Foundation Fellowship. Generous financial support was also received from the BACHE FUND

of the NATIONAL ACADEMY OF SCIENCES and the Penrose Fund of the American Philosophical Society. To these organizations the writer is deeply grateful.

² Mr. William Greenwood, a resident of Lautoka, Viti Levu, and an enthusiastic amateur botanist, has made several excursions to the western slopes of the Mt. Evans Range and has ascended the subsidiary southernmost peak, Mbotilamu, which is only slightly lower than Koroyanitu. As a result of Mr. Greenwood's excellent collections, which in recent years have been sent to the Arnold Arboretum for study, several new species of flowering plants were discovered on and near the Mt. Evans Range, including *Elatostema Greenwoodii*, *Dysoxylum pilosum*, *Pterocymbium oceanicum* and *Tapeinosperma Greenwoodii*. The unusual features of Mr. Greenwood's collections first called the writer's attention to this neglected area and gave it priority when the opportunity to pursue field work was offered.

³ Although representative specimens were obtained from all the plants mentioned, their exact botanical identification must await detailed study of the entire collection.

⁴ Pickering Peak of the Europeans, and the second isolated forested range of western Viti Levu. An ascent of this was subsequently made by the writer; it is somewhat lower than Koroyanitu, but is more isolated and offers a more difficult climb.

NUTRITIONAL LIFE HISTORY AS INFLUENCED BY DIETARY ENRICHMENTS. III. FULL-LIFE DATA OF 1946-1948 EXPERIMENTS*

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In previous papers of this series^{1, 2} we have reported that starting with a basal diet of natural foods containing 14.4 per cent of protein and 0.2 per cent of calcium, with laboratory-bred rats as experimental animals, supplementation with meat protein results in more rapid growth and earlier reproduction, but occasionally at some cost to stability. Usually but not always there has been spontaneous recovery.

Such experiments have now been extended to larger numbers; and, in the cases started earliest, to the completion of the life histories.

Table 1 summarizes the average findings for all of the completed full-life studies with the two diets chiefly used in our earliest-started experiments. Comparing the first two columns of figures in this table it will be seen that the larger numbers of findings fully confirm our previous report of more rapid growth and earlier puberty in the animals receiving the protein supplement. These animals also reared somewhat more and larger young. Their distinctly lesser average age at death is doubtless due to the greater incidence of premature deaths in early adulthood.

■ The last 3 columns of table 1 give the average finding of 5 comparisons of litter mates receiving, respectively (1) the basal Diet A (Diet 16) alone

(2) Diet 16 P 5 consisting of Diet 16 plus a supplement of 5 g. of meat six days each week, and (3) Diet 16 P 10 containing twice as large a meat (protein) supplement. This was a series in which no premature deaths occurred, and the lengths of life on the three diets were essentially alike. In other respects also, the two levels of meat feeding (protein supplementation) yielded practically the same results.

TABLE 1

AVERAGE RECORDS OF FEMALE RATS ON DIET 16 ALONE OR WITH PROTEIN (MEAT) SUPPLEMENT: COMPLETED LIVES

	ALL COMPARABLE CASES ON THESE TWO DIETS		FIVE CASES EACH LITTER-MATE PARALLELS		
	16 CASES ON DIET 16	19 CASES ON DIET 16 P 5	ON DIET 16	ON DIET 16 P 5	ON DIET 16 P 10
Gain in body wt., 28th-56th days of life, g.	43	60	37	62	62
Age at birth of first young, days	121	107	120	100	94
Number of young borne, per female	40	41	30	45	53
Number of young reared, per female	28	34	24	35	43
Average wt. of young at 28 days, g.	39	44	40	46	43
Age at death, days	820*	668†	846	849	905

* One of the 16 of this series died before 400 days of age.

† Four of the 19 of this series died before 400 days of age.

While the figures in table 1 are believed to give a valid general impression, they are not offered as a precise measure either of the incidence of "premature" deaths or of the effect of protein supplementation. Moreover, as pointed out in our previous papers^{1, 2} the occasional unfavorable reactions are very largely prevented by increasing the calcium content of the diets receiving the extra protein. Experiments to determine the effect of such calcium supplementation are still in progress, and these with their controls will also increase the volume of data from which to judge the occasional suggestions of imbalance.

TABLE 2

EARLY GROWTH AND LENGTH OF LIFE OF MALE RATS ON DIETS 16, 16 P 5 AND 16 P 10

	10 CASES ON DIET 16	10 CASES ON DIET 16 P 5	6 CASES ON DIET 16 P 10
Gain in body wt., 28th-56th days of life, g.	49	82	78
Age at death, days	741	748	711

Table 2 summarizes the data of growth and length of life of the males corresponding to the females represented in table 1. The protein supplementation clearly increased the rate of growth in the males and to about

the same proportionate degree as in the females. The length of life was not significantly influenced in either direction. There were no "premature" deaths among the 26 male rats used in these experiments. Nor have we observed in any of these males the symptoms of excessive nervousness which occasionally appeared among the females as previously described.

* Aided by grants from the John and Mary R. Markle Foundation to Columbia University.

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ON THE PROTEINS OF a^+a^+ AND aa EPHESTIA

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It has been shown previously that lack or reduction of pigment in a number of organs in *Ephestia* homozygous for the gene a is due to lack of kynurenin, a precursor of the pigment.¹ Kynurenin is formed from tryptophane in the metabolism of a^+ -animals.* In aa moths the tryptophane content of the proteins is higher than in the wild type.² Since no concomitant increase in protein nitrogen was found, it was concluded that either a new type of protein is formed under the influence of the gene a , or that proteins rich in tryptophane replaced others poor in tryptophane. In either case, the protein make-up of the cell would be changed.

Since proteins perform important functions in the cell, it should be expected that other characters of the cell are also changed. Actually it has been found that fat content,^{2, 3} viability,⁴ speed of development⁴ and possibly O_2 consumption of homogenates⁵ are reduced in aa animals. It has been concluded that as a result of the mutation $a^+ \rightarrow a$ the biochemical make-up of the cell is changed in several respects.

The characters influenced by the genes a^+ and a are summarized in table 1. Included in this table are the effects of a third allele a^k as far as they are known. Besides differing in a number of pigmentation characters, a^+ and a differ from each other in chemical composition (kynurenin content, tryptophane content, fat content), and in some general biological characteristics such as viability and speed of development. The gene a^k appears intermediate between a^+ and a , as far as pigmentation characters are concerned. The same appears to be true for kynurenin content, since $a^k a^k$ mothers were able to pass on a substance capable of darkening larval ocelli to their $a^k a$ offspring, and since the same maternal effect was observed in aa

offspring from *aa* mothers into which degenerating $a^k a^k$ ovaries had been grafted.⁶ The speed of development, on the other hand, is still more reduced in $a^k a^k$ than in *aa*. With regard to pigmentation characters and kynurenin formation, the series of genes would be $a^+ > a^k > a$, while with regard to speed of development they would be arranged in the order $a^+ > a > a^k$. Similar differences in serial arrangement in multiple alleles according to the characters observed have been repeatedly found. The remaining characters have not been investigated in $a^k a^k$.

It appears difficult to decide in which physiological order the different characters observed should be arranged. It was proved by injection experiments¹ that the pigment reduction in *aa* is due to lack of kynurenin. A

TABLE 1
PLEIOTROPIC EFFECTS OF THE GENES a^+ , a^k AND *a*

A. Pigmentation characters	$a^+ a^+$	$a^k a^k$	<i>aa</i>
1. Eyes	Black	Brown	Red
2. Brain	Brown	Light pink
3. Testes	Pigmented	Weakly pigmented—colorless	Colorless
4. Larval ocelli	Strong pigmentation	Intermediate pigmentation	Weak pigmentation
5. Larval hypodermis	Pigmented	Colorless	Colorless
6. Proteins	Pink	White
B. Chemical constitution			
7. Kynurenin	Present	Reduced in amount	Strongly reduced or absent
8. Protein tryptophane	?	Increased
9. Ether-extractable substances	?	Reduced
C. General biological characteristics			
10. Viability		Reduced	Reduced
11. Speed of development		Strongly reduced	Reduced
12. O ₂ consumption in homogenates		?	Reduced ?

block of the oxidation of tryptophane to kynurenin may be considered to be the primary effect of the gene *a*. The non-oxidized tryptophane would then be stored secondarily in part in the proteins which are consequently qualitatively changed. The reduction in fat content, in viability and in speed of development may be assumed to be a consequence of the altered protein constitution. In $a^k a^k$, the amount of kynurenin seems to be smaller than in a^+ , but larger than in *aa*. The proteins of $a^k a^k$ may therefore be expected to be intermediate in tryptophane content. Unfortunately, this assumption could not be investigated. It can be imagined, however, that the resulting $a^k a^k$ proteins have a more profound action on speed of development than

the *aa* proteins. Considerations of this kind may explain the frequent occurrence of non-seriability found in multiple alleles.

An alternative assumption would be that the formation of certain proteins rich in tryptophane is the primary step caused by the gene *a*. In this case tryptophane bound up in the proteins would not be available for kynurenin formation. Again the change in fat content, etc., may be another consequence of the changed protein constitution. If the difference in protein constitution were the primary effect of the gene *a*, causing less tryptophane to be available for the formation of kynurenin, it might be expected that *aa* proteins might release tryptophane at a slower rate than *a⁺a⁺* proteins under the influence of proteolytic enzymes. With this possibility in mind, autolysis was studied in *a⁺a⁺* and *aa* homogenates. In the experiments, fully grown *a⁺a⁺* and *aa* larvae were used. The *aa* strain had been crossed out previous to the experiment for 8 and 9 consecutive generations to the *a⁺a⁺* strain used, so that the alleles were compared on a reasonably isogenic background. 2.5-3.0 g. larval material were finely ground in homogenizers according to Potter and Elvehjem in 3 ml. Ringer solution isotonic for *Ephestia* and buffered with 0.02 *M* phosphate buffer to pH 6.8. The larvae were homogenized at 0°C. for 10 minutes. In the homogenates, total tryptophane was determined according to the method of Sullivan and Hess,⁷ and total nitrogen by a micro Kjeldahl procedure. One ml. of the homogenate was pipetted into each one of 5 small test tubes and incubated in a water bath at 31°C. The homogenate was examined for non-protein nitrogen and for non-protein tryptophane at 0, 1, 2, 4 and 6 hours after the start of the experiment. 0.1 ml. of 3 *M* trichloroacetic acid was added to each tube, and the precipitate filtered off and washed with 0.4 ml. H₂O. In the filtrate, tryptophane and nitrogen were determined. Seven paired experiments with both *a⁺a⁺* and *aa* homogenates and one experiment using *a⁺a⁺* material only were run.

At time 0, there are 8 measurements of tryptophane and nitrogen for *a⁺a⁺*, and 7 for *aa* which can be used to indicate the tryptophane content of *a⁺a⁺* and *aa* larval material. The data are given in tables 2a and b. In

TABLE 2a
TRYPTOPHANE AND NITROGEN CONTENT IN HOMOGENATES OF ISOGENIC *a⁺a⁺* AND *aa*
EPHESTIA IN MG./G. WET WEIGHT

STRAIN	TOTAL N	NON-PROTEIN N	TOTAL TRYPTOPHANE	NON-PROTEIN TRYPTOPHANE
<i>a⁺a⁺</i>	13.8	1.49	1.22	0.04
<i>aa</i>	14.5	1.43	1.42	0.10

table 2a, the values for protein and non-protein nitrogen and tryptophane are given, expressed in relation to wet weight. Qualitatively, the data indicate an increase in total and non-protein tryptophane in *aa* as compared

to a^+a^+ without a concomitant increase in nitrogen. For a quantitative analysis, these data are unsuited because of the variability of both nitrogen and tryptophane in relation to wet weight. Quantitative data for tryptophane in relation to nitrogen content are given in table 2b.

TABLE 2b

RATIOS OF TRYPTOPHANE AND NITROGEN IN THE TOTAL AND NON-PROTEIN FRACTION OF a^+a^+ AND aa EPHESTIA HOMOGENATES

	a^+a^+	aa	t	$P_{df=18}$
Total tryptophane Total N	0.088	0.098	2.251	< 0.05
Non-protein tryptophane Non-protein N	0.027	0.070	3.241	< 0.01
Non-protein N Total N	0.108	0.099	0.068	> 0.5
Non-protein tryptophane Total tryptophane	0.033	0.070	3.315	< 0.01

The ratio total tryptophane/total nitrogen is increased in aa as compared a^+a^+ . The difference is slightly below the 0.05 level of significance, i.e., just on the borderline of statistical significance. It appears, however, that this difference is real, in view of previously published results,⁷ in which it has been found both for aa imagoes and aa larvae that the tryptophane content as related to dry weight is increased in comparison with a^+a^+ animals. Furthermore, the second line of table 2b shows that the non-protein tryptophane expressed in terms of non-protein nitrogen is higher in aa than a^+a^+ homogenates. This difference is well below the 0.01 level of significance. It should be pointed out that in the data involving non-protein tryptophane the level of significance calculated is probably too high, since 2 of the 8 values for a^+a^+ were given as 0, while they may have contained amounts of tryptophane too small to be measured. On the other hand, differences in the same direction and of the same order of magnitude have been found previously for aa and a^+a^+ imagoes, so that the conclusion appears justified that in aa material the amount of non-protein tryptophane is increased. No separate values for protein tryptophane are available in the present series of experiments. The tryptophane values in table 2a, columns 3 and 4, indicate, however, that the absolute amounts of non-protein tryptophane (0.04 and 0.10 mg./g. wet weight) are too small to account for the whole difference in total tryptophane content between a^+a^+ and aa material, 0.16 mg./g. wet weight. It must be concluded that part of the increased tryptophane content of aa is stored in the proteins, in agreement with earlier direct findings in imagoes. On the other hand, the ratio non-protein tryptophane/total tryptophane is almost twice as large

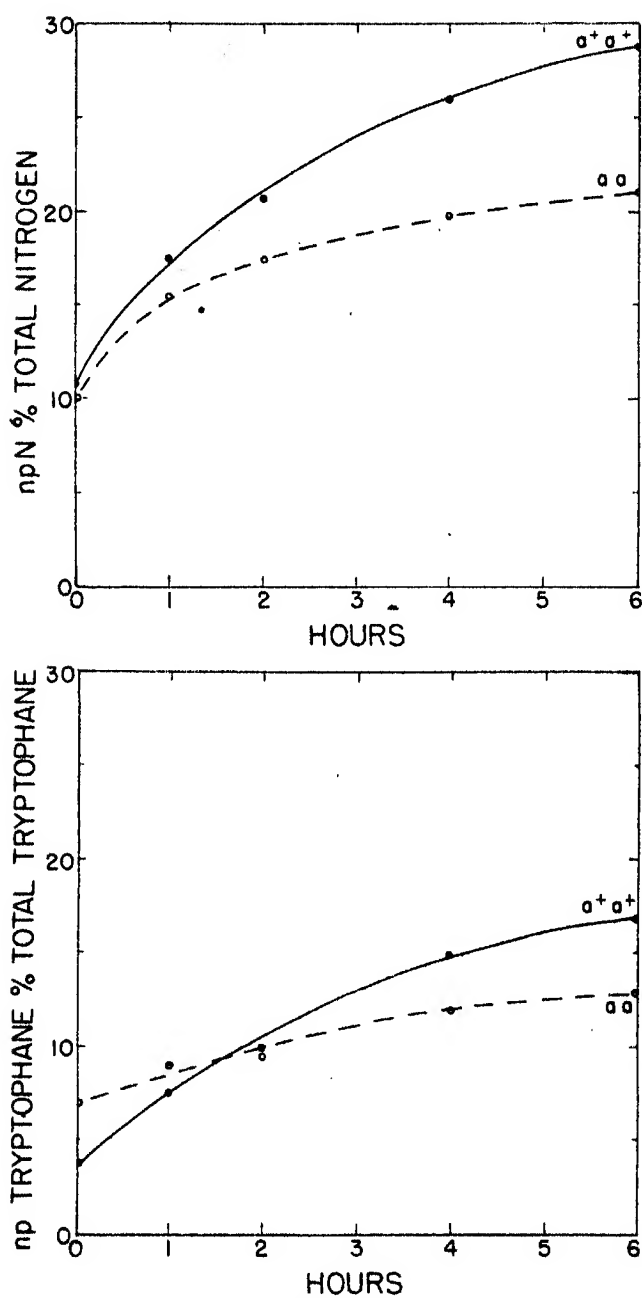


FIGURE 1

Rate of autolysis of homogenates from a^+a^+ and aa Ephestia: (a) release of non-protein nitrogen; (b) release of non-protein tryptophane.

in *aa* than in *a⁺a⁺* larvae; a higher proportion of the tryptophane is found in the non-protein fraction in *aa* than in *a⁺a⁺* material.

The results of the autolysis experiments are recorded in figure 1. In these curves non-protein nitrogen and non-protein tryptophane are expressed as per cent of the total nitrogen and total tryptophane, respectively. The points are the means of the different determinations. It should be emphasized that while the curves obtained in the individual experiments vary considerably, the relative rate of release of nitrogen and tryptophane in the two strains is consistent in all paired experiments.

The rate of autolysis appears to be very high, compared with other autolysis experiments. This may be due to the fact that old larvae nearing pupation were used in the experiments. It must be assumed that potent proteolytic enzymes are active during the breakdown of larval tissues at metamorphosis, and these enzymes may start to be produced in fully grown larvae.

There are decided differences between the rates of release of both non-protein nitrogen and non-protein tryptophane in the two strains. Both substances increase more rapidly in *a⁺a⁺* than in *aa* material. The amounts of non-protein nitrogen in the two strains are originally identical. After 6 hours, the non-protein nitrogen has increased by 17.8% of the total nitrogen in *a⁺a⁺* material while in *aa* homogenate it increased only by 11% of the total nitrogen. The percentage of non-protein tryptophane is originally higher in *aa* than *a⁺a⁺* material, 7.0% as against 3.3% of the total tryptophane. The rate of increase in non-protein tryptophane under the influence of autolytic enzymes is again slower in *aa* than in *a⁺a⁺* material, increasing in 6 hours by 6.75% in *aa* as against 13.5% of the total tryptophane in *a⁺a⁺* homogenate.

The difference in the rate of autolysis may be due either to differences in the structure of the proteins or in the activity of the enzymes concerned. In either case, it would agree with the previous observation that the protein make-up of the cell is changed under the influence of the gene *a*. If the differences in rate of autolysis are due to a different resistance of the proteins to the same enzyme system, the possibility would not be excluded that the primary action of the gene *a* is the formation of different proteins richer in tryptophane and more resistant to autolysis. Both these facts would tend to reduce the amount of free tryptophane available for kynurenin formation. However, the fact that the relative amount of non-protein tryptophane is found to be increased in *aa* animals would tend to favor the alternative that the primary effect of the gene *a* is a block of the oxidation of tryptophane to kynurenin.

If tryptophane were bound in different types of linkage in *a⁺a⁺* and *aa* proteins, it might possibly be released during autolysis at different rates in relation to nitrogen. Material concerning this question is given in table 3

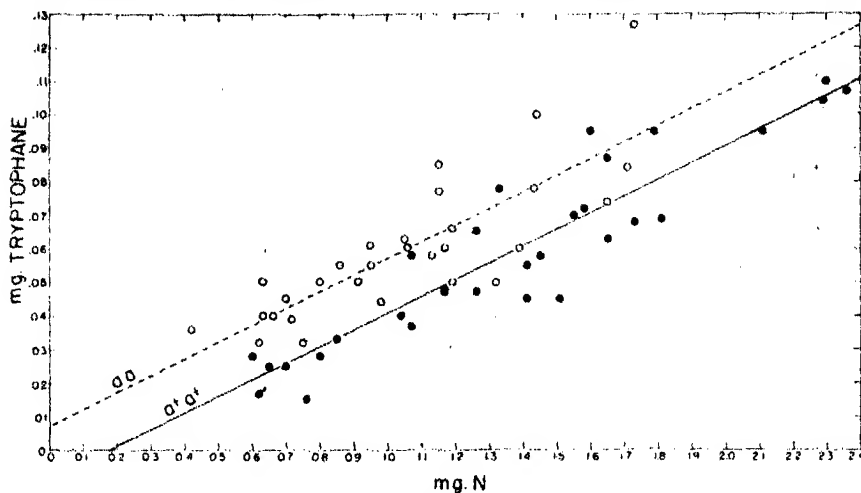


FIGURE 2
Correlation between non-protein nitrogen and non-protein tryptophane: (a) in a^+a^+ homogenates; (b) in aa homogenates. The lines represent the regression equations.

and figure 2. In figure 2, the corresponding tryptophane and nitrogen values for the same samples are plotted in a scatter diagram. If tryptophane were released at a faster rate, relative to nitrogen, in one strain than in the other, the slopes of the two curves would be different. Actually the slopes for the two strains are almost identical, the linear regression coefficients being 0.0496 and 0.0497 mg. tryptophane per mg. N, respectively. The closeness of the association of the different values found with the linear regression line and consequently the variability of the values is indicated by the correlation coefficient. The last values on table 3 indicate that the correlation coefficients for the two strains are not significantly different. All these findings give no reason to suspect that tryptophane is bound in a different way in aa than in a^+a^+ proteins.

TABLE 3
RELATION BETWEEN NON-PROTEIN NITROGEN AND NON-PROTEIN TRYPTOPHANE IN THE COURSE OF AUTOLYSIS IN a^+a^+ AND aa EPHESTIA HOMOGENATES

STRAIN	NO. OF DETERMINATIONS	REGRESSION COEFFICIENTS MG. TRYPTOPHANE/MG. N	CORRELATION COEFFICIENT
a^+a^+	30	0.0497	0.923 ± 0.027
aa	29	0.0496	0.817 ± 0.062

In view of the great number of physiological differences found between a^+a^+ and aa animals the question appears legitimate whether physiological differences of the same order of magnitude may be associated with other genes. While no direct evidence on this point is available the frequent

occurrence of characteristic effects of genes on viability⁸ and on shape of the spermatheca⁹ in *Drosophila* seem to suggest that this is the case.

Summary.—The tryptophane content of *aa* *Ephestia* larvae is higher than that of *a⁺a⁺* *Ephestia* larvae in the non-protein fraction and probably also in the protein fraction. In homogenates of isogenic *a⁺a⁺* and *aa* homogenates, autolysis proceeds at a faster rate in *a⁺a⁺* than in *aa* material. Formation under the influence of the gene *a* of a protein richer in tryptophane and more resistant to proteolytic enzymes would constitute a conceivable mechanism for inhibition of kynurenin formation in *aa* animals.

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THE RAYLEIGH-RITZ AND THE WEINSTEIN METHODS FOR APPROXIMATION OF EIGENVALUES. II. DIFFERENTIAL OPERATORS

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1. In the present paper we are going to apply the results of our first paper¹ to eigenvalue problems for differential operators. In general, the problems will be of the following type. Given two linear (ordinary or partial) differential operators A and B , A of higher degree than B , the operators being defined for functions in some domain D , we consider the equation

$$Au = \theta Bu, \quad \theta \text{ a constant parameter.} \quad (1)$$

We want then to find functions satisfying in the domain D the equation (1) and on the boundary C of D some homogeneous boundary conditions which will be denoted by (B) .

The problem is to find the values of the parameter θ for which such functions $u \neq 0$ exist. In the usual cases, when the problem is well defined, it will admit a discreet sequence of eigenvalues θ_k , and our aim will be to apply the results of our previous paper to the computation of these eigenvalues.

2. To this effect we have to transform the differential problem into a problem concerning a completely continuous operator in a Hilbert space. To do this we follow the already classical method of transforming the differential problem (1) into an equivalent variational problem concerning the minimum or maximum of the expression

$$\frac{\mathfrak{A}(u)}{\mathfrak{B}(u)} \quad (2)$$

for all functions u defined in the domain D and satisfying the boundary conditions (B) .² The expressions $\mathfrak{A}(u)$ and $\mathfrak{B}(u)$ are quadratic functionals, usually integro-differential forms in the function u . They are chosen in such a way that equation (1) be the Euler equation of the variational problem. It is possible to do this only when the operators A and B are self-adjoint differential operators. In this case, for the simplest kind of boundary conditions, it is possible to take the \mathfrak{A} and \mathfrak{B} as given by the formulae

$$\mathfrak{A}(u) = \int_D A u \cdot u \, d\omega, \quad \mathfrak{B}(u) = \int_D B u \cdot u \, d\omega.$$

We will have to suppose further that the form $\mathfrak{A}(u)$ is positive definite.

In many cases this form can be transformed (by use of boundary conditions) into a formally positive form, for example:

$$\int_D -\Delta u \cdot u \, d\omega = \int_D [u_x^2 + u_y^2] \, d\omega, \text{ for the boundary condition } u = 0,$$

$$\int_D \Delta \Delta u \cdot u \, d\omega = \int_D |\Delta u|^2 \, d\omega, \text{ for the boundary condition } u = \frac{\partial u}{\partial n} = 0.$$

For two differential problems, with the same equation (1) but with different boundary conditions (B) and (B') , the corresponding variational problem may deal with the same quadratic forms $\mathfrak{A}(u)$ and $\mathfrak{B}(u)$. But when the differential problems are well defined, the classes of admissible functions are different—no one being contained in the other.

3. We now translate the variational problem into language of the Hilbert space.³ To this effect we consider the class \mathcal{K} of admissible functions, i.e., the class of functions for which the operators A and B are defined, the quadratic forms \mathfrak{A} and \mathfrak{B} are finite and the boundary conditions (B) are satisfied.

In this class the quadratic definite positive form $\mathfrak{A}(u)$ defines a norm

$\|u\| = \sqrt{\mathfrak{H}(u)}$ and a scalar product $\mathfrak{H}(u, v)$, which we will denote as usual in the Hilbert space by (u, v) .

The class \mathcal{K} does not yet form a Hilbert space because it is not complete. By a functional completion⁴ we get from \mathcal{K} a class of functions $\overline{\mathcal{K}}$ forming a Hilbert space. Usually the form $B(u)$ may be extended on the whole of $\overline{\mathcal{K}}$ and will form there a completely continuous quadratic form (the degree of B being smaller than that of A).

By a classical theorem of Fréchet-Riesz, the quadratic form \mathfrak{B} gives rise to a symmetric completely continuous operator K such that

$$\mathfrak{B}(u) = (Ku, u). \quad (3)$$

Further, we will have the equality

$$AKu = Bu \quad \text{when } AKu \text{ exists.} \quad (4)$$

As we have already noticed, for two variational problems which differ only in the boundary conditions, say (B) and (B') , and which concern the same expression (2), the classes of admissible functions are never included one in another. In spite of this, it might very well happen that if we pass from classes \mathcal{K} and \mathcal{K}' to the complete spaces $\overline{\mathcal{K}}$ and $\overline{\mathcal{K}'}$, we get the inclusion $\overline{\mathcal{K}} \subset \overline{\mathcal{K}'}$. The explanation of this lies in the fact that some boundary conditions are stable and others unstable in respect to the operation of completion for a given quadratic form \mathfrak{H} . This means that some boundary conditions will remain valid for all functions of the completed class whereas others will not.

For instance, take the quadratic form $\mathfrak{H}(u) = \int |\Delta u|^2 d\omega$ and, as boundary conditions (B) , consider $u = \Delta(u) = 0$ on the boundary C , and as boundary conditions (B') : $u = \frac{\partial u}{\partial n} = 0$ on C . It can be proved that when we complete the class \mathcal{K} the only remaining boundary condition will be $u = 0$, but when we complete the class \mathcal{K}' , both the boundary conditions remain valid (the second, $\frac{\partial u}{\partial n} = 0$, in a weakened form).

As can be seen in this example, we will have in general $\overline{\mathcal{K}} \subset \overline{\mathcal{K}'}$, if all the boundary conditions of \mathcal{K}' which are not satisfied by \mathcal{K} are unstable.

We will now consider the eigenvalue problem for the operator K which is of the type considered in our first paper. As before, we consider the variational problem concerning the expression

$$\frac{(Ku, u)}{(u, u)} = \frac{\mathfrak{B}(u)}{\mathfrak{H}(u)} \quad (5)$$

which is the inverse of the expression (2). Consequently, the eigenvalues

of K are the inverses of the eigenvalues of our variational problem (2). The eigenfunctions u_n will satisfy the equation

$$Ku_n = \lambda_n u_n. \quad (6)$$

In order that these eigenfunctions be solutions of our differential problem, we have to make a hypothesis which is usually satisfied, in that there exists a positive integer γ such that

$$K^\gamma u \in \mathcal{K} \quad \text{for every } u \in \overline{\mathcal{K}}, \quad (7)$$

and since we then have for u_n (by (4)), $AK^\gamma u_n = BK^{\gamma-1} u_n$: $Au_n = \theta_n Bu_n$, with $\theta_n = \frac{1}{\lambda_n}$, and as $K^\gamma u_n = \lambda_n^\gamma u_n \in \mathcal{K}$, u_n satisfies equation (1) and all the boundary conditions.

By making some other hypotheses which are usually satisfied in the cases which have been considered, we also prove, conversely, that every solution of the differential eigenvalue problem is an eigenfunction for our operator K with the corresponding eigenvalue $\lambda_n = \frac{1}{\theta_n}$.

4. The basic theorem which allows us to apply the methods of Rayleigh-Ritz and Weinstein to the differential problems is the following:

THEOREM P. *If two variational problems of type (2), with the same quadratic forms \mathfrak{A} and \mathfrak{B} but different classes \mathcal{K} and \mathcal{K}' give rise to complete spaces $\overline{\mathcal{K}}$ and $\overline{\mathcal{K}'}$ with $\overline{\mathcal{K}} \subset \overline{\mathcal{K}'}$, the operator K is the part of K' in $\overline{\mathcal{K}}$.*

This theorem shows the importance of completing the classes \mathcal{K} and \mathcal{K}' by functions. If we completed them by abstract elements we would not be able to compare the classes $\overline{\mathcal{K}}$ and $\overline{\mathcal{K}'}$.

We will now illustrate the application of the Weinstein and generalized Rayleigh-Ritz methods by a few examples.

We will consider, in particular, the problem of eigenvalues of a clamped plate. The differential problem is the following one:

$$\Delta^2 u = \theta u, \quad \mathcal{K}: u = \frac{\partial u}{\partial n} = 0 \text{ on } C. \quad (8)$$

5. We will first apply Weinstein's method by considering another problem with the same equation but with boundary conditions given by

$$\mathcal{K}': u = \Delta u = 0 \text{ on } C. \quad (8')$$

This second problem is easily seen to be equivalent to the problem of vibrations of a membrane, namely, the problem $\Delta u + \sqrt{\theta} u = 0$ in D , $u = 0$ on C .

The two problems lead to variational problems with the same forms \mathfrak{A} and \mathfrak{B} , namely

$$\begin{aligned}\mathfrak{A}(u) &= \int_D |\Delta u|^2 d\omega, \\ \mathfrak{B}(u) &= \int_D |u|^2 d\omega.\end{aligned}\quad (9)$$

The complete class $\overline{\mathfrak{K}}$ is the class of all functions $u(z)$:

$$u(z) = \int_D g(z, z') f(z') d\omega', \quad (10)$$

where g is the ordinary Green's function of the domain D , $f = -\Delta u$ and f is in square integrable in D . The boundary condition $u = 0$ is maintained (is stable), but the condition $\Delta u = 0$ is lost (unstable). The complete class $\overline{\mathfrak{K}}$ is a subclass of $\overline{\mathfrak{K}'}$, and as already has been said, the condition $u = 0$ is stable and the $\frac{\partial u}{\partial n} = 0$ is also stable in a weakened form. The latter condition is equivalent to a condition first introduced by S. Zaremba,⁵ in that $(u, p) = 0$ for all $p \in \overline{\mathfrak{K}'}$ harmonic of second order (i.e., $\Delta^2 p = 0$). With $f = -\Delta u$, $h = -\Delta p$, it means $\int_D f h d\omega = 0$ for all h harmonic and in square integrable. If \mathcal{O} is the class of all the p , it follows that $\overline{\mathfrak{K}'} \ominus \overline{\mathfrak{K}} = \mathcal{O}$.

We can now give the expressions for the operators K' and K . We introduce the functions

$$g_2(z, z') = \int_D g(z, z'') g(z'', z') d\omega'' \quad (11)$$

(the Green's function for $\Delta^2 u = 0$, $u = \Delta u = 0$ on C),

$$g_{II}(z, z') = \text{Green's function for } \Delta^2 u = 0, u = \frac{\partial u}{\partial n} = 0 \text{ on } C. \quad (12)$$

As functions of z (for z' fixed), $g_2 \in \overline{\mathfrak{K}'}$ and $g_{II} \in \overline{\mathfrak{K}}$. We have then

$$K'u = \int_D g_2(z, z') u(z') d\omega', \quad u \in \overline{\mathfrak{K}'}, \quad (13)$$

$$Ku = \int_D g_{II}(z, z') u(z') d\omega', \quad u \in \overline{\mathfrak{K}}. \quad (14)$$

For both operators K and K' , the integer γ of (7) is $= 1$ and we have

$$\text{for } u \in \overline{\mathfrak{K}'}: K'u \in \overline{\mathfrak{K}'}, \quad \Delta^2 K'u = u, \quad (15)$$

$$\text{for } u \in \overline{\mathfrak{K}}: Ku \in \overline{\mathfrak{K}}, \quad \Delta^2 Ku = u. \quad (16)$$

Now, if we know the complete solution of the problem of vibrations of a membrane for a domain D , we can take the class $\overline{\mathfrak{K}'}$ as the starting class for Weinstein's method and, by a choice of a sequence, $\{p_k\} \in \mathcal{O}$, we may calculate approximate values for the eigenvalues of K , i.e., the eigenvalues of the clamped plate. This is how Weinstein himself proceeded in his investigations.⁶ He applied the method to the case of a rectangle where the membrane problem is explicitly solved.

But the method may be applied in a much more general case. We have only to change our starting class. Instead of changing the boundary

conditions and maintaining the same domain D , we can maintain the boundary conditions by taking a domain D_0 larger than D . This means that we will consider the problem of a clamped plate in the domain D_0 . We shall have to suppose then that the domain D_0 is chosen so that we know explicitly the solutions of the clamped plate problem. For instance, we can take D_0 to be a circle. We will then consider the quadratic forms \mathfrak{A} and \mathfrak{B} in D_0 as well as in D , and we will consider the class $\overline{\mathfrak{K}}$ for the domain D and $\overline{\mathfrak{K}}_0$ for the domain D_0 . If the function u of $\overline{\mathfrak{K}}$ is continued in the whole domain D_0 by putting $u(z) = 0$, for $z \in D_0 - D$, it becomes a function defined in D_0 and belonging to $\overline{\mathfrak{K}}_0$, as is easily proved. In this way the class $\overline{\mathfrak{K}}$ may be considered as contained in $\overline{\mathfrak{K}}_0$. It forms there a closed linear subspace and for the functions of $\overline{\mathfrak{K}}$ we see immediately that

$$\mathfrak{A}(u) = \mathfrak{A}_0(u), \quad \mathfrak{B}(u) = \mathfrak{B}_0(u).$$

It follows again that the operator K is the part of K_0 in $\overline{\mathfrak{K}}$. We can then apply again the Weinstein method to approximate the eigenvalues of K . To do this we have to consider the subspace $\overline{\mathfrak{K}}_0 \ominus \overline{\mathfrak{K}}$. It can be proved that this subspace is generated by a sequence $g_{II}^0(z, z_n)$ for any sequence $\{z_n\}$ dense in the domain $D_0 - D$, g_{II}^0 being the function g_{II} corresponding to the domain D_0 . If we take a finite number of functions $g_{II}^0(z, z_1), \dots, g_{II}^0(z, z_n)$ and consider them as the functions p_k in the Weinstein method, we can form Weinstein's determinant $W(\xi)$ and it is easily proved that this determinant is given by the formula

$$W(\xi) = \left(\frac{-1}{\xi}\right)^n \det. \left\{ g_{II}^0\left(z_i, z_k, \frac{1}{\xi}\right) \right\},$$

where $g_{II}^0(z, z', \xi)$ is the Green's function in the domain D_0 of $\Delta^2 u - \xi u = 0$, $u = \frac{\partial u}{\partial n} = 0$ on the boundary. When D_0 is a circle, this latter Green's function can be computed with the help of Bessel functions and we get in this way the possibility of computing lower bounds for the eigenvalues of clamped plates of any shape. The more the points z_1, \dots, z_n are dense in $D_0 - D$, the better the lower bounds will be.

6. The applications of the ordinary Rayleigh-Ritz method are well known and very often used. This method will give upper bounds for a finite number of eigenvalues. To get upper bounds for all the eigenvalues we have to apply the generalized Rayleigh-Ritz method.

To show an example of application of the generalized Rayleigh-Ritz method consider two domains D and D_0 in the same way as in the last example of Weinstein's method, only now D_0 will be contained in D . We consider again the problem of vibrations of a clamped plate in both these domains. By a device used above, we will find that now the class $\overline{\mathfrak{K}}_0$ is

a subclass of \mathcal{K} and if we take for D_0 a domain where the clamped plate problem is explicitly solvable, we will be in a position to apply the generalized Rayleigh-Ritz method.

As it may seem difficult in the present case to calculate the determinant $D(\zeta)$ and even to establish an explicit complete sequence $\{p_k\}$ in the subspace $\mathcal{K} \ominus \overline{\mathcal{K}}_0$ we shall indicate briefly how this is to be done. We will take for D_0 a circle.

First, we take a complete sequence of functions $\{q_k(z)\}$ in the class $\overline{\mathcal{K}}$. This can be done in different ways. In the case when the boundary of D is given by an equation $\beta(z) \equiv \beta(x, y) = 0$ with β twice continuously differentiable in the closed domain \bar{D} we can take for $\{q_k(z)\}$ the sequence of all functions $x^m y^n (\beta(z))^2$, $m, n = 0, 1, 2, \dots$

Then the projections p_k of q_k on the subspace $\mathcal{K}_1 = \mathcal{K} \ominus \overline{\mathcal{K}}_0$ will form a complete sequence in \mathcal{K}_1 . The function $p_k(z)$ is readily seen to be $= q_k(z)$ in $D - D_0$ and $= h_k(z)$ in D_0 , where h_k is the harmonic function of order 2 ($\Delta \Delta h_k = 0$) in D_0 such that on the boundary C_0 of D_0 $h_k = q_k$ and $\frac{\partial h_k}{\partial n} = \frac{\partial q_k}{\partial n}$.

Now we have to calculate the function $p_k^{(0)} = P_0 K p_k$, where P_0 is the projection on $\overline{\mathcal{K}}_0$ and K is the operator given by (14) in the class $\overline{\mathcal{K}}$. In spite of the fact that K is not explicitly known (the function g_{II} for D is not known) we can calculate $p_k^{(0)}$ in the following way: $p_k^{(0)}$ is the solution of the equation

$$\Delta \Delta p_k^0 = p_k = h_k \text{ in } D_0, \quad p_k^0 = \frac{\partial p_k^0}{\partial n} = 0 \text{ on } C_0.$$

We have then to calculate the functions $w_k(\zeta) \equiv w_k(z, \zeta) = R_\zeta^{(0)} p_k^{(0)}$. By the properties of the operator K_0 , these functions appear as the solutions of the equation

$$w_k - \zeta \Delta \Delta w_k + h_k = 0 \text{ in } D_0, \quad w_k = \frac{\partial w_k}{\partial n} = 0 \text{ on } C_0.$$

w_k as a function of ζ is a transcendental function which can always be written (by the use of spectral decomposition) as an infinite sum of simple fractions in ζ .

The general term of the determinant $D(\zeta)$ is $(K w_k + K p_k - \zeta p_k, p_l)$. By the definition of the scalar product in $\overline{\mathcal{K}}$ and the properties of the operator K , we obtain this term in the following form

$$\int_D (w_k + p_k) p_l d\omega - \zeta \int_D \Delta p_k \Delta p_l d\omega.$$

This shows already the possibility of calculating the determinant $D(\zeta)$ and of applying the generalized Rayleigh-Ritz method.

Before concluding the present paper, let us remark that the methods apply in a similar manner to differential operators in more than two variables.

¹ These PROCEEDINGS, 34, 474-480 (1948).

² Courant-Hilbert, *Methoden der Math. Physik*, vol. I, Chap. 6, and Vol. II, Chap. 7.

³ This translation has already been used, especially by K. Friedrichs (e.g., in *Math. Ann.*, 109, pp. 465 and 685, (1934) and in more recent papers), also by J. W. Calkin (*Trans. Am. Math. Soc.*, 45, p. 369 (1939)).

⁴ For the definition of a functional completion cf. N. Aronszajn, *Comptes Rendus Ac. Sc. Paris*, 226, p. 537 (1948).

⁵ Zaremba, S., *Ann. Sc. Ec. Norm. Sup.*, 26 (1909).

⁶ Weinstein, A., *Memorial des Sciences Math.*, 88 (1937).

ON THE THEORY OF AGE-DEPENDENT STOCHASTIC BRANCHING PROCESSES

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We are interested in investigating the following mathematical problem which is of possible biological, chemical and physical interest. A particle existing at time $t_1 = 0$ is assumed to have a probability q_n , $n \geq 1$, of being transformed into n similar particles at some random time $t > 0$. Under the hypotheses that any particle has a life-length independent of its time of birth and the number of other particles existing at the time, and that there is no death, we require the probability distribution of $Z(t)$, the number of particles in existence at time t .

In our work, it is assumed that the random transformation times have a cumulative distribution $G(t)$, where $G(0) = 0$, and $G(\infty) = 1$. Depending upon the depth of the result we wish to prove, further assumptions are added. The most general of our results are derived under the assumption that $G(t)$ has a derivative $g(t)$, a density function, which is itself of bounded variation over any finite interval, and satisfies a slight additional restriction.

There has been a large amount of research done on the corresponding problem where the transformation time is independent of the age of the particle, cf., e.g., Harris,³ Kolmogoroff,⁴ Kolmogoroff and Sevastyanov,⁵ Sevastyanov,⁶ Yaglom.⁷ To the best of the authors' knowledge, the problem in this paper has not been considered previously, although probabilities have been allowed to depend upon absolute time, cf. Arley,¹ where other references are given. Proofs of the results communicated here and further details will be published elsewhere at a later time.

A central rôle, as usual, is played by the generating function

$$F(s, t) = \sum_{r=1}^{\infty} P_r(t) s^r, \quad |s| \leq 1, \quad (1)$$

where

$$P_0(t) = \text{Prob} (Z(t) = r \text{ at time } t), \quad r \geq 1. \quad (2)$$

Let as above q_n , $n \geq 1$, be the probability that when the particle is transformed, it is transformed into n particles, and let $h(s) = \sum_{n=1}^{\infty} q_n s^n$. Standard probabilistic arguments yield the non-linear integral equation

$$F(s, t) = \int_0^t h(F(s, t-y)) dG(y) + s(1 - G(t)). \quad (3)$$

Non-linearity is a characteristic feature of the age-dependent theory as contrasted with the linearity of the Markoff processes of age-independent theory.

A routine application of the method of successive approximations yields

THEOREM 1. *Under the sole assumptions*

$$dG \geq 0, \quad G(0) = 0, \quad G(\infty) = 1, \quad (4)$$

equation (3) has for $|s| \leq 1$ a unique bounded solution possessing the elementary properties of a generating function, namely

$$F(s, t) = \sum_{r=1}^{\infty} P_r(t) s^r, \quad P_r(t) \geq 0, \quad |s| \leq 1, \quad (5a)$$

$$F(1, t) \equiv 1, \quad (5b)$$

$$F(s, 0) = s, \quad P_1(t) = \left. \frac{\partial F}{\partial s} \right|_{s=0} = 1 - G(t). \quad (5c)$$

Furthermore, the moments

$$m_k(t) = \sum_{n=1}^{\infty} n^k P_n(t) \quad (6)$$

exist for $0 \leq t < \infty$, $k = 1, 2, \dots$, provided that the moments $\sum_{n=1}^{\infty} n^k q_n$ exist for $k = 1, 2, \dots$.

The explicit form of the $P_r(t)$ may now be determined by means of the above.

It is now of interest to investigate the distribution of the random variable

$$w(t) = Z(t)/E(Z(t)), \quad (7)$$

where $E(Z(t))$ is the expectation of Z , the first moment $m_1(t)$. The results

we obtain are generalizations of those of Harris,³ and Yaglom,⁷ derived under the assumption of age independence. We require first the asymptotic behavior of $E(Z(t))$. It is easy to show that the expectation satisfies the linear integral equation

$$u(t) = c_1 \int_0^t u(t-y) dG(y) + (1 - G(t)), \quad (c_1 = h^1(1)), \quad (8)$$

which we recognize as the familiar linear equation of renewal theory.

The asymptotic behavior of the solutions of this equation has been extensively investigated by Feller.² From his results, we obtain

THEOREM 2. *Under the previous assumptions concerning $G(t)$, and the further assumptions that*

$$G(y) = \int_0^y g(u) du, \quad \int_0^\infty e^{-by} |dg| < \infty \quad (9a)$$

for some $b > 0$, whose value depends upon c_1 , and

$$1 < c_1 = \sum_{n=1}^{\infty} n q_n < \infty, \quad (9b)$$

we obtain the asymptotic relations

$$m_k \sim a_k e^{kat}, \quad k = 1, 2, \dots, \quad (10)$$

as $t \rightarrow \infty$, where a is defined as the positive root of

$$1 = c_1 \int_0^\infty e^{-ay} dG(y), \quad (11)$$

provided that

$$\sum_{n=1}^{\infty} n^k q_n < \infty, \quad k = 1, 2, \dots \quad (12)$$

The result of (10) is also derived under other assumptions on $G(t)$ which permit it to have a step-function behavior. These results require more involved methods, and as they are of lesser importance, we shall not discuss them here.

Applying the results of Theorem 2, we derive the most important result of our researches

THEOREM 3. *Under the assumptions of Theorems 1 and 2, the random variable $w(t)$ converges in probability to a random variable w .*

Let $K(t)$ be the cumulative of \bar{w} , where $\bar{w} = Z(t)/e^{at}$, and set

$$\phi(s) = \int_0^\infty e^{-sy} dK(y). \quad (13)$$

It follows that

$$\phi(s) = \lim_{t \rightarrow \infty} F(s/e^{at}, t) \quad (14)$$

and that

$$\phi(s) = \int_0^\infty h(\phi(s/e^{ay}))dG(y). \quad (15)$$

$K(t)$ is a continuous function of t . If we further assume that

$$\int_{t_1}^\infty g(y)dy \leq e^{-bt}, \quad t \geq t_1 \quad (16)$$

for some $b > 0$, then $K(t)$ is differentiable, so that $\phi(s)$ has the form

$$\phi(s) = \int_0^\infty e^{sy}k(y)dy. \quad (17)$$

From (16) we also derive bounds on the magnitude of $|\phi(it)|$ as $t \rightarrow \pm \infty$. These estimates are of value in computing $k(y)$ using the Fourier inversion formula.

These results are susceptible of generalization in several important directions. One may consider the case where death occurs, or the still more general case where death occurs and the probability of splitting is dependent on the time of birth. Then there are the problems of the distribution of ages, the number of splits in a given time interval, and so on. Finally, there is the case where there are particles of different types which give birth not only to those of the same type, but to those of other types. Biological mutation is included in this case. We hope to discuss these problems subsequently. Let us note that in many important situations a suitable transformation reduces the case with death to a case without death.

Finally, we may mention that several of the methods of the present paper are contained in essence in a paper by one of the authors, Harris.²

¹ Arley, N., *On the Theory of Stochastic Processes and Their Application to the Theory of Cosmic Radiation*, Copenhagen, 1943.

² Feller, W., "The Integral Equation of Renewal Theory," *Ann. Math. Statistics*, **12**, 243-268 (1943).

³ Harris, T. E., "Branching Processes," *Ibid.*, to appear.

⁴ Kolmogoroff, A., "Branching Stochastic Processes," *Compt. rend. (Doklady)*, **56**, 5-8 (1947) (Russian).

⁵ Kolmogoroff, A., and Sevastyanov, B., "On the Asymptotic Form of the Probability for Stochastic Branching Processes," *Akad. Nauk (Doklady)*, **56**, 783-787 (1947) (Russian).

⁶ Sevastyanov, B., "On the Theory of Stochastic Branching Processes," *Ibid.*, **59**, 1407-1409 (1948) (Russian).

⁷ Yaglom, A., "Some Theorems in the Theory of Stochastic Branching Processes," *Ibid.*, **56**, 795-799 (1947) (Russian).

CONFORMAL MAPPING AND CONVERGENCE OF A POWER SERIES

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It is well known that there exist power series which map their circle of convergence onto a simply-connected domain D and which converge at every point of the boundary of this circle. For example, such is the case if the boundary of D is a Jordan curve; this follows from a theorem by Fejér.¹

The present paper is devoted to the following question: do there exist power series with the above-mentioned convergence property, which map their convergence circle onto the universal covering surface of a multiply-connected domain D ?

In answering this question we prove two lemmas (placed at the end of the paper) which, it is believed, have some interest for their own sake.

By way of introduction consider, for example, a power series $w = w(z) = \sum_{n=0}^{\infty} a_n z^n$ mapping the unit circle C , $|z| < 1$, onto the universal covering surface S of the circular ring $1/2 < |w| < 1$. Consider on S all the straight lines which join the points $1/2$ and 1 . Their images on C form a doubly infinite sequence of analytic curves (c_n) , $n = \dots, -1, 0, +1, \dots$, having the following properties: 1° each curve c_n is situated in the interior of C and joins two different boundary points, 2° two different curves of (c_n) never have a point in common, 3° every continuous curve situated in C and joining a point of c_{n-1} with a point of c_{n+1} must intersect c_n , and 4° the curves (c_n) tend to the boundary of C as $n \rightarrow \infty$ and as $n \rightarrow -\infty$. The three first properties are evident, the fourth is seen in the following way.

Let z_n be the point (or one of the points) of c_n which has the largest distance from the boundary of C . The sequence (z_n) has at least one accumulation point z' , and z' must be located on the boundary of C , for, otherwise, its image would be an interior point of S , and this is impossible. Hence $\lim_{n \rightarrow \pm \infty} |z_n| = 1$ by the above property 3°.

Now consider in C the radius vector from the origin to the point $z' = e^{i\varphi}$. This radius intersects an infinite number of curves c_n , and so we can choose a sequence of points $r_k e^{i\varphi}$, $k = 1, 2, \dots$, lying on it such that 1° $r_k < r_{k+1}$, $k = 1, 2, \dots$, 2° $\lim_{k \rightarrow \infty} r_k = 1$, and 3° $w(r_{2k-1} e^{i\varphi}) > 1/2$ and $w(r_{2k} e^{i\varphi}) < -1/2$, $k = 1, 2, \dots$. Therefore, $\lim_{r \rightarrow 1-0} w(re^{i\varphi})$ cannot exist, and, by Abel's theorem, $\sum a_n e^{in\varphi}$ cannot converge.

Now, consider the more general case in which $\sum a_n z^n$ maps C onto the

universal covering surface of an arbitrary multiply-connected bounded domain D . The boundary of D then consists of an exterior contour B and a remainder B' consisting of one or more interior contours or points. By an argument analogous to the one above, we see that, if B' consists of more than one point, then $\sum a_n z^n$ must diverge at two boundary points at least.

Our only chance then to construct a power series satisfying the desired convergence property is to choose as D a bounded domain, whose boundary consists of an exterior contour and one isolated point.

The Taylor series $\sum_{n=0}^{\infty} c_n z^n$ of the function² $w = \exp \frac{z+1}{z-1}$, where we have written $\exp a$ for e^a , has the above-mentioned mapping property. In fact, it is easily verified: 1° that this series has the convergence radius one, 2° that the function $u = \frac{z+1}{z-1}$ maps the unit circle of the z -plane onto the half-plane $Re u < 0$ of the u -plane and the point $z = 1$ into the point $u = \infty$, and 3° that the function $w = e^u$ maps the half-plane $Re u < 0$ onto the universal covering surface of the circle $|w| < 1$ punctured at $w = 0$. Here, the point $u = \infty$ corresponds in a certain sense to the point $w = 0$. The function $w = \exp \frac{z+1}{z-1}$ has of course an essential singularity at $z = 1$ with the Picard exceptional values 0 and ∞ .

The coefficient c_n of the power series

$$w = \exp \frac{z+1}{z-1} = \sum_{n=0}^{\infty} c_n z^n, \quad |z| < 1, \quad (1)$$

can be written in the form

$$c_n = \frac{1}{2\pi i} \int_{C_\epsilon} \exp \frac{z+1}{z-1} \frac{dz}{z^{n+1}} \quad (2)$$

where C_ϵ is the boundary of the common part of the domains $|z| < 1$ and $|z-1| > \epsilon$, $0 < \epsilon < 1$. Now the function $\exp \frac{z+1}{z-1}$ is bounded for $|z| \leq 1$ and $z \neq 1$, and hence we can let $\epsilon \rightarrow 0$ in (2), giving

$$c_n = \frac{1}{2\pi i} \int_{|z|=1} \exp \frac{z+1}{z-1} \frac{dz}{z^{n+1}}.$$

Making the variable transformation $z = e^{i\theta}$ and observing that c_n is real, we get

$$\begin{aligned}
 c_n &= \frac{1}{2\pi} \int_{-\pi}^{\pi} \exp \frac{e^{i\theta} + 1}{e^{i\theta} - 1} \frac{d\theta}{e^{in\theta}} = \frac{1}{2\pi} \int_{-\pi}^{\pi} \left\{ \cos \left(\cot \frac{\theta}{2} \right) \right. \\
 &\quad \left. - i \sin \left(\cot \frac{\theta}{2} \right) \right\} \left\{ \cos n\theta - i \sin n\theta \right\} d\theta = \frac{1}{2\pi} \int_{-\pi}^{\pi} \cos \left(\cot \frac{\theta}{2} \right) \\
 &\quad \cos n\theta d\theta - \frac{1}{2\pi} \int_{-\pi}^{\pi} \sin \left(\cot \frac{\theta}{2} \right) \sin n\theta d\theta = a_n - b_n,
 \end{aligned}$$

let us say.

We write

$$a_n = \frac{1}{\pi} \int_0^{\pi} \cos \frac{2}{\theta} \cos n\theta d\theta + \frac{1}{\pi} \int_0^{\pi} \left\{ \cos \left(\cot \frac{\theta}{2} \right) - \cos \frac{2}{\theta} \right\} \cos n\theta d\theta = a'_n + a''_n.$$

Here the series $\sum a'_n$ converges by Lemma 1. The coefficient a''_n is the n th cosine coefficient of the Fourier series of the even periodic function $g(\theta)$, which coincides in $(0, \pi)$ with the function

$$\begin{aligned}
 \frac{1}{2} \left\{ \cos \left(\cot \frac{\theta}{2} \right) - \cos \frac{2}{\theta} \right\} &= \frac{1}{2} \left\{ \cos \left(\frac{2}{\theta} + 0(\theta) \right) - \cos \frac{2}{\theta} \right\} = \\
 &= -\sin \left(\frac{2}{\theta} + 0(\theta) \right) \sin 0(\theta). \quad (3)
 \end{aligned}$$

Now the above-mentioned Fourier series converges to zero for $\theta = 0$ by Dini's test, for the function $g(\theta)/\theta$ is integrable in $(0, \pi)$, as is easily seen by the last expression (3). Hence the series $\sum a''_n$ is convergent.

Analogously we write

$$b_n = \frac{1}{\pi} \int_0^{\pi} \sin \frac{2}{\theta} \sin n\theta d\theta + \frac{1}{\pi} \int_0^{\pi} \left\{ \sin \left(\cot \frac{\theta}{2} \right) - \sin \frac{2}{\theta} \right\} \sin n\theta d\theta = b'_n + b''_n$$

and observe that the series $\sum b'_n$ converges by Lemma 2. Here b''_n is the n th cosine coefficient of the conjugate series of the Fourier series of the odd periodic function $h(\theta)$, which coincides in $(0, \pi)$ with

$$\begin{aligned}
 \frac{1}{2} \left\{ \sin \left(\cot \frac{\theta}{2} \right) - \sin \frac{2}{\theta} \right\} &= \frac{1}{2} \left\{ \sin \left(\frac{2}{\theta} + 0(\theta) \right) - \sin \frac{2}{\theta} \right\} = \\
 &= \cos \left(\frac{2}{\theta} + 0(\theta) \right) \sin 0(\theta).
 \end{aligned}$$

Hence the conjugate series converges for $\theta = 0$ by Dini's test, since the function $h(\theta)/\theta$ is integrable in $(0, \pi)$. Therefore the series $\sum b''_n$ is convergent.

Summing up our results we find that the series (1) converges for $z = 1$. And its sum is zero by Abel's theorem, since, writing $z = re^{i\theta}$, we have $\lim_{r \rightarrow 1-0} w = 0$, where w is defined by (1). Further it is evident that the series (1) converges for every fixed z with $|z| = 1$ and $z \neq 1$ to a value with modulus one.

Thus we can state our main result in the following way:

The power series (1) maps the interior of the unit circle onto the universal covering surface of the domain $0 < |w| < 1$ of the w -plane and it converges at every point of the boundary of its convergence circle, though not uniformly.

LEMMA 1. Consider the Fourier series of the even periodic function $g(\theta)$ which in $(0, \pi)$ coincides with $\cos \frac{2}{\theta}$:

$$g(\theta) \sim \frac{1}{2} a_0 + \sum_{n=1}^{\infty} a_n \cos n\theta.$$

This series converges to zero for $\theta = 0$.

Proof: It is sufficient³ to show that

$$\int_0^1 \frac{g(\theta) \sin n\theta}{\theta} d\theta \rightarrow 0 \text{ as } n \rightarrow \infty. \quad (4)$$

We write

$$\begin{aligned} \int_0^1 \cos \frac{2}{\theta} \frac{\sin n\theta}{\theta} d\theta &= \frac{1}{2} \int_0^1 \sin \left(n\theta - \frac{2}{\theta} \right) \frac{d\theta}{\theta} + \frac{1}{2} \int_0^{\sqrt{2/n}} \sin \left(n\theta + \frac{2}{\theta} \right) \\ &\quad \frac{d\theta}{\theta} + \frac{1}{2} \int_{\sqrt{2/n}}^1 \sin \left(n\theta + \frac{2}{\theta} \right) \frac{d\theta}{\theta} = \frac{1}{2} (P + Q + R). \end{aligned} \quad (5)$$

In the integral P we make the substitution $n\theta - 2/\theta = t$, $\theta = (t + \sqrt{t^2 + 8n})/2n$ and we find

$$P = \int_{-\infty}^{n-2} \frac{\sin t}{\sqrt{t^2 + 8n}} dt = \int_{-\infty}^0 + \int_0^{n-2}.$$

The second mean-value theorem now gives

$$P = \frac{1}{\sqrt{8n}} \int_0^{\tau} \sin t dt + \frac{1}{\sqrt{8n}} \int_0^{\tau'} \sin t dt,$$

where $-\infty < \tau < 0$ and $0 < \tau' < n - 2$, and so

$$|P| \leq \frac{1}{\sqrt{8n}}. \quad (6)$$

To estimate Q we use the substitution $n\theta + 2/\theta = t$, $\theta = (t - \sqrt{t^2 - 8n})/2n$, and we obtain

$$Q = \int_{\sqrt{8n}}^{\infty} \frac{\sin t}{\sqrt{t^2 - 8n}} dt.$$

Choose the integer p such that $(p-1)\pi < \sqrt{8n} < p\pi$. Then

$$Q = \left[\int_{\sqrt{8n}}^{p\pi} + \sum_{k=1}^{\infty} \int_{(p+k-1)\pi}^{(p+k)\pi} \right] \frac{\sin t}{\sqrt{t^2 - 8n}} dt = u + \sum_{k=1}^{\infty} \sum u_k,$$

say. Here $\sum u_k$ is an alternating series whose terms decrease monotonically in absolute value, and so the modulus of its sum is less than $|u_1|$. Hence

$$|Q| < |u| + |u_1| < \int_{\sqrt{8n}}^{(p+1)\pi} \frac{|\sin t|}{\sqrt{t^2 - 8n}} dt < \int_{\sqrt{8n}}^{\sqrt{8n} + 2\pi} \frac{dt}{\sqrt{t^2 - 8n}} < \frac{1}{2\sqrt{2n}} \int_{\sqrt{8n}}^{\sqrt{8n} + 2\pi} \frac{dt}{\sqrt{t - \sqrt{8n}}}$$

or

$$|Q| < \frac{\sqrt{2\pi}}{\sqrt{2n}}. \quad (7)$$

Finally, to get an upper bound for $|R|$, we set $n\theta + 2/\theta = t$, $\theta = (t + \sqrt{t^2 - 8n})/2n$ and obtain

$$R = \int_{\sqrt{8n}}^{n+2} \frac{\sin t}{\sqrt{t^2 - 8n}} dt.$$

But this expression can be estimated in just the same way as Q ; the only difference is that the series $\sum u_k$ now has only a finite number of terms. Hence we also have

$$|R| < \frac{\sqrt{2\pi}}{\sqrt{2n}}. \quad (8)$$

Summing up the results (6), (7) and (8) we see that the desired relation (4) holds, and Lemma 1 is proved.⁴

LEMMA 2. Consider the Fourier series of the odd periodic function $h(\theta)$ which in $(0, \pi)$ coincides with $\sin \frac{2}{\theta}$:

$$h(\theta) \sim \sum_{n=1}^{\infty} b_n \sin n\theta. \quad (9)$$

Then the series $\sum_{n=1}^{\infty} b_n$ is convergent.

Proof: Here b_n is the n th cosine coefficient of the conjugate series of the series (9), and we have to show that this conjugate series converges for $\theta = 0$. To this end it is sufficient⁵ to prove the three relations:

$$\int_0^t h(\theta) d\theta = o(t) \text{ as } t \rightarrow +0, \quad (10)$$

$$\lim_{\epsilon \rightarrow +0} \int_{\epsilon}^1 h(\theta) \frac{d\theta}{\theta} \text{ exists,} \quad (11)$$

and

$$\int_{1/n}^1 h(\theta) \frac{\cos n\theta}{\theta} d\theta \rightarrow 0 \text{ as } n \rightarrow \infty. \quad (12)$$

Formula (10) is immediate:

$$\int_0^t \sin \frac{2}{\theta} d\theta = \int_0^t \left\{ \sin \frac{2}{\theta} + \theta \cos \frac{2}{\theta} \right\} d\theta + o(t) = \frac{t^2}{2} \cos \frac{2}{t} + o(t).$$

The statement (11) is easily verified by the substitution $1/\theta = t$. Finally to demonstrate (12), we write

$$\begin{aligned} \int_{1/n}^1 \sin \frac{2 \cos n\theta}{\theta} d\theta &= -\frac{1}{2} \int_{1/n}^1 \sin \left(n\theta - \frac{2}{\theta} \right) \frac{d\theta}{\theta} + \\ &\quad \frac{1}{2} \int_{1/n}^{\sqrt{2/n}} \sin \left(n\theta + \frac{2}{\theta} \right) \frac{d\theta}{\theta} + \frac{1}{2} \int_{\sqrt{2/n}}^1 \sin \left(n\theta + \frac{2}{\theta} \right) \frac{d\theta}{\theta}. \end{aligned}$$

These terms can be treated in the same way as the corresponding terms of (5), giving (12), and Lemma 2 is proved.

¹ *Compt. rend., Paris*, **156**, p. 46 (1913). Montel, *Leçons sur les familles normales*, Paris, 1927, p. 119. Further examples are given in the author's article, "Sur la convergence des séries potentielles," *Arkiv Mat., Astron., Fysik, Uppsala*, **31B**, no. 6 (1944).

² After submitting this paper I have learned that Pringsheim (*Sitzber. bayer. Akad., Math. u. Phys. Klasse*, p. 92 (1900)) has studied the convergence properties of the Taylor series of $\exp \frac{z}{z-1}$, though from a point of view different from the one adopted here.

³ See Hardy-Rogosinski, *Fourier Series*, Cambridge, 1944, p. 38, Theorem 50.

⁴ In proving Lemma 1 we have used Cauchy integrals instead of Lebesgue integrals in some instances. This can easily be avoided in the following way: use ϵ , $0 < \epsilon < \sqrt{2/n}$, as lower bound in the integral (4), and repeat the whole argument giving

$$\left| \int_{\epsilon}^1 \cos \frac{2 \sin n\theta}{\theta} d\theta \right| < \frac{1}{\sqrt{2n}} + 2 \frac{\sqrt{2\pi}}{\sqrt[4]{2n}}; \text{ now let } \epsilon \rightarrow 0.$$

⁵ See Hardy-Rogosinski, *loc. cit.*, p. 48, Theorem 61.

ESSENTIAL MULTIPLICITY AND LEBESGUE AREA

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1. *Introduction.*—This note exhibits some applications of standard methods of algebraic topology to the theory of area.

We suppose that M is a k -dimensional orientable manifold, X is a compact locally connected subset of M , Y is k -dimensional Euclidean space and C is the set of all continuous functions on X to Y , which is metrized by the function d such that

$$d(f, g) = \sup_{x \in X} |f(x) - g(x)| \quad \text{for } f, g \in C.$$

For $f \in C$ and each open subset U of Y we define $F(f, U)$ to be the family of all components of the set

$$X \cap E[f(x) \in U].$$

We observe that if f, U are as above and $y \in U$, then there are at most finitely many $A \in F(f, U)$ for which $A \cap E[f(x) = y]$ is non-vacuous.

Let $H^n(A, B)$ be the n -dimensional Čech cohomology group of A modulo B with integer coefficients, whenever $A \supset B$ are compact.

We recall that if $A \supset B$ are compact subsets of a k -dimensional manifold for which $A - B$ is non-vacuous and connected, then $H^k(A, B)$ is infinite cyclic or trivial according as B does or does not contain the boundary of A .

Let L_k be the k -dimensional Lebesgue measure over Y .

2. *Definition of the Multiplicity.*—If $f \in C$, U is an open connected subset of Y with compact closure and $A \in F(f, U)$, then $D(f, U, A)$ is the non-negative integer defined as follows: In case $H^k(\text{Clos } A, \text{Bdry } A)$ is infinite cyclic, the homomorphism of $H^k(\text{Clos } U, \text{Bdry } U)$ into $H^k(\text{Clos } A, \text{Bdry } A)$ induced by $(f|_{\text{Clos } A})$ relates to a generator of the first group an integral multiple of a generator of the second group, and $D(f, U, A)$ is defined as the absolute value of the multiplier, which is independent of the choice of generators.¹ In case $H^k(\text{Clos } A, \text{Bdry } A)$ is trivial, we define $D(f, U, A) = 0$.

If f and U are as above, then the set of all those $A \in F(f, U)$ for which the f image of A contains U is finite. Hence $D(f, U, A) = 0$ for all but finitely many $A \in F(f, U)$, and we define the non-negative integer

$$D(f, U) = \sum_{A \in F(f, U)} D(f, U, A).$$

It is readily seen that if $f \in C$ and $U_1 \supset U_2$ are open connected subsets of Y with compact closure, then $D(f, U_1) \leq D(f, U_2)$.

For each $y \in Y$ we define $M(f, y)$, the essential multiplicity with which f assumes the value y , as the supremum of $D(f, U)$ for all open connected neighborhoods U of y with compact closure. Since $D(f, U)$ is monotone with respect to U on the set of all these neighborhoods partially ordered by inclusion, \supset , we have

$$M(f, y) = \lim_{n \rightarrow \infty} D(f, U_n)$$

whenever $U_1 \supset U_2 \supset U_3 \supset \dots$ are any such neighborhoods whose intersection contains only y .

It is easy to check that if $f, g \in C$ and $U_1 \supset U_2$ are open connected subsets of Y with compact closures such that

$$d(f, g) \leq \text{distance}(U_2, Y - U_1),$$

then

$$D(g, U_2) \geq D(f, U_1).$$

It follows that the function M is lower semicontinuous on the cartesian product space $(C \times Y)$.

3. *Approximation Theorems.*—The Hopf extension theorem yields the following information:

If $f \in C$, U is an open convex cell with compact closure contained in Y , $A \in F(f, U)$ and $D(f, U, A) = 0$, then there is a $g \in C$ such that

$$\begin{aligned} g(x) &= f(x) && \text{for } x \in X - A, \\ g(x) &\in \text{Bdry } U && \text{for } x \in A. \end{aligned}$$

If f, U, A are as above, but $D(f, U, A) > 0$, and if $y \in U$, then there is a $g \in C$ and an open cell B such that $\text{Clos } B \subset A$,

$$\begin{aligned} g(x) &= f(x) && \text{for } x \in X - A, \\ g(x) &\in \text{Bdry } U && \text{for } x \in A - B, \\ g(x) &\in U && \text{for } x \in B, \\ (g|_{\text{Clos } B}) &\text{ is simplicial,} \\ A \cap_x E[g(x) = y] &\text{ has one element,} \\ A \cap_x E[g(x) = z] &\text{ has } D(f, U, A) \text{ elements} \end{aligned}$$

for L_z almost all z in U .

Furthermore the cell B may be prescribed.

Applying the preceding two lemmas to all those $A \in F(f, U)$ for which $A \cap_x E[f(x) = y]$ is non-vacuous, and a modification by central projection

from y onto $\text{Bdry } U$ to all other $A \in F(f, U)$, we prove:

If $f \in C$, U is an open convex cell with compact closure contained in Y , $y \in U$, and n is the number of those $A \in F(f, U)$ for which $D(f, U, A) \neq 0$, then there exist a map $g \in C$ and open cells B_1, \dots, B_n , whose closures are contained in distinct elements of $F(f, U)$, such that

$g(x) = f(x)$ whenever $f(x) \in Y - U$,

$g(x) \in \text{Bdry } U$ whenever $f(x) \in U$ and not $x \in \bigcup_{j=1}^n B_j$,

$g(x) \in U$ whenever $x \in \bigcup_{j=1}^n B_j$,
 $\left(g|_{\bigcup_{j=1}^n \text{Clos } B_j}\right)$ is simplicial,

$E[g(x) = y]$ has n elements,

\times

$E[g(x) = z]$ has $D(f, U)$ elements for L_k almost all z in U .

\times

This theorem concerning the modification of a mapping on the counterimage of a single cell, may be applied at once to modify a map on the counterimage of the union of any finite set of disjoint cells. This yields the following proposition:

If $f \in C$ and U_1, \dots, U_m are those k -cells of a polyhedral subdivision of Y which meet range f , then there exist a map $g \in C$ and open cells B_1, \dots, B_n ,

whose closures are contained in distinct elements of $\bigcup_{i=1}^m F(f, U_i)$, such that

$g(x) = f(x)$ whenever $f(x) \in Y - \bigcup_{i=1}^m U_i$,

$g(x) \in \text{Bdry } U_i$ whenever $f(x) \in U_i$ and not $x \in \bigcup_{j=1}^n B_j$,

$g(x) \in U_i$ whenever $f(x) \in U_i$ and $x \in B_j$,

$\left(g|_{\bigcup_{j=1}^n B_j}\right)$ is simplicial,

$E[g(x) = z]$ has $D(f, U_i)$ elements for L_k almost all z in U_i .

g maps $X - \bigcup_{j=1}^n B_j$ into the $k - 1$ skeleton of the polyhedral subdivision of Y .

If, in addition, X is a polyhedron, then B_1, \dots, B_n may be chosen as cells of a refinement of any given polyhedral subdivision of X , and for each positive number ϵ there is a simplicial map $h \in C$ such that $d(g, h) < \epsilon$,

$$g(x) = h(x) \quad \text{for } x \in \bigcup_{j=1}^n B_j,$$

h maps $X - \bigcup_{j=1}^n B_j$ into the $k - 1$ skeleton of the subdivision of Y .

If diameter $U_i < \epsilon$ for $i = 1, \dots, m$, then $d(f, h) < \epsilon$.

4. Application to Area.—Let G be the function on C such that

$$G(f) = \int_Y M(f, y) dL_k y \quad \text{for } f \in C.$$

The lower semicontinuity of M and Fatou's lemma imply that the Gebeze area G is lower semicontinuous on C .

If $f \in C$ and P is a finite family of disjoint open connected subsets of Y with compact closures, then

$$\sum_{U \in P} D(f, U) \cdot L_k(U) \leq G(f),$$

because $D(f, U) \leq M(f, y)$ for $y \in U \in P$.

The fact that $G(f)$ is actually the supremum of such sums is a consequence of the following proposition:

If $f \in C$ and P_1, P_2, P_3, \dots are such finite families of disjoint open connected subsets of Y with compact closures that

$$\begin{aligned} \lim_{n \rightarrow \infty} \sup_{U \in P_n} \text{diameter } U &= 0, \\ \lim_{n \rightarrow \infty} L_k(\text{range } f - \bigcup_{U \in P_n} U) &= 0, \end{aligned}$$

then

$$\lim_{n \rightarrow \infty} \sum_{U \in P_n} D(f, U) \cdot L_k(U) = G(f).$$

To prove this by contradiction we may assume, after passage to a subsequence if necessary, that

$$\lim_{n \rightarrow \infty} \sum_{U \in P_n} D(f, U) \cdot L_k(U) < G(f),$$

$$\sum_{n=1}^{\infty} L_k(\text{range } f - \bigcup_{U \in P_n} U) < \infty,$$

whence

$$L_k\left[\bigcap_{m=1}^{\infty} \bigcup_{n=m}^{\infty} (\text{range } f - \bigcup_{U \in P_n} U)\right] = 0.$$

We define

$$r(U, y) = 1 \text{ for } y \in U, r(U, y) = 0 \text{ for } y \in Y - U,$$

and observe that

$$\lim_{n \rightarrow \infty} \sum_{U \in P_n} D(f, U) \cdot r(U, y) = M(f, y)$$

for $y \in \text{range } f - \bigcap_{m=1}^{\infty} \bigcup_{n=m}^{\infty} (\text{range } f - \bigcup_{U \in P_n} U)$, hence for L_k almost all y in $\text{range } f$.

Now Fatou's lemma implies

$$\lim_{n \rightarrow \infty} \sum_{U \in P_n} D(f, U) \cdot L_k(U) = \lim_{n \rightarrow \infty} \int_{\text{range } f} \sum_{U \in P_n} D(f, U) \cdot r(U, y) dL_k y \geq G(f).$$

This contradiction completes the proof.

If X is a polyhedron, $f \in C$ and $\epsilon > 0$, we may choose P as the set of all those k -cells of a polyhedral subdivision of Y which meet range f and so that diameter $U < \epsilon$ for $U \in P$. Then the last theorem of Section 3 yields a simplicial map $h \in C$ for which $d(f, h) < \epsilon$ and

$$G(h) = \sum_{U \in P} D(f, U) \cdot L_k(U) \leq G(f).$$

We conclude that if X is a polyhedron, then G is the Lebesgue area.

For the special case in which $k = 2$ and X is a 2-cell this result is substantially due to Lamberto Cesari, who has also very ingeniously solved the corresponding much deeper problem for maps of a 2-cell into 3-space.² The extension to maps of k -cells into n -space, with $2 \leq k < n$ and $n > 3$, appears still remote.

5. *Essential Components and Stable Multiplicity.*—If $f \in C$, $y \in Y$ and T is a component of $E[f(x) = y]$, then T is said to be *essential* if and only

x

if $D(f, U, A) > 0$ whenever U is an open connected neighborhood of y with compact closure and $T \subset A \in F(f, U)$.

Let $E(f, U)$ be the number of those $A \in F(f, U)$ for which $D(f, U, A) > 0$, and let $S(f, y)$ be the supremum of $E(f, U)$ with $y \in U$. Clearly $S(f, y) \leq M(f, y)$.

We observe that if $U_1 \supset U_2$ and $D(f, U_1) < D(f, U_2)$, then $E(f, U_1) < E(f, U_2)$. Hence $M(f, y) = \infty$ if and only if $S(f, y) = \infty$.

The essential components are all sets of the type

$$\bigcap_{n=1}^{\infty} A_n, \text{ where } A_1 \supset A_2 \supset A_3 \supset \dots, D(f, U_n, A_n) > 0,$$

$U_1 \supset U_2 \supset U_3 \supset \dots$ and $\bigcap_{n=1}^{\infty} U_n = \{y\}$. Hence $S(f, y)$ is the number of essential components of $E[f(x) = y]$.

x

The function S is lower semicontinuous on $(C \times Y)$.

Let $N(f, y)$ be the number (possibly ∞) of x for which $f(x) = y$. Clearly $S(f, y) \leq N(f, y)$. Our first approximation theorem implies

$$S(f, y) = \liminf_{g \rightarrow f} N(g, y),$$

so that $S(f, y)$ is the stable multiplicity with which f assumes y .

For the special case in which $k = 2$ and X is a closed 2-cell these results are substantially due to Rado and Reichelderfer.³ The function S has also been used by the author.⁴

Cesari has shown,⁵ by a method depending significantly on the existence of covering maps of the punctured plane with prescribed degree, that if

$k = 2$, X is a 2-cell and $f \in C$, then the set of all those y for which $S(f, y) < M(f, y)$ is countable. However, if $k > 2$, this inequality may hold on a set of positive measure; for instance f may wrap a solid cube several times around an arc of positive volume. Hence the results of the preceding section indicate that M is more suitable than S for the theory of area.

¹ Here Bdry A refers to the relative topology of X .

² Cesari, L., "Una uguaglianza fondamentale per l'area delle superficie," *Atti della Reale Accademia d'Italia, Memorie*, 14, 891-951 (1944).

³ Rado, T., and Reichelderfer, P. V., "A Theory of Absolutely Continuous Transformations in the Plane," *Trans. Am. Math. Soc.*, 49, 258-307 (1941).

⁴ Federer, H., "Coincidence Functions and Their Integrals," *Ibid.*, 59, 441-466 (1946).

⁵ Cesari, L., "Sui punti di diramazione delle trasformazioni continue e sull'area delle superficie in forma parametrica," *Univ. Roma e Ist. Naz. Alla Mat. Rend. Mat. e Appl.*, ser. 5, 3, 37-62 (1942).

SOME NEW RESULTS ON PARTITIONS

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This paper contains the statements of five theorems on partitions, the proofs of which will appear in a series of papers in another American journal, devoted entirely to mathematics.

Euler's identity

$$\frac{1}{(1-x)(1-x^2)(1-x^3)\cdots} = (1+x)(1+x^2)(1+x^3)\cdots \quad (1)$$

may be paraphrased as follows:

The number of partitions of n into odd parts is equal to the number of partitions of n into distinct parts.

Definition 1: The *rank* of a partition is the excess of the maximum part over the number of parts.¹

Definition 2: $D_r(n)$ = the number of partitions of n into distinct parts, the rank of each partition being r .

Definition 3: $U_{2r+1}(n)$ = the number of partitions of n into odd parts, the maximum part being $2r+1$.

It is clear that the rank of a partition into distinct parts cannot be negative. Hence Euler's theorem may be written

$$U_1(n) + U_3(n) + U_5(n) + \cdots = D_0(n) + D_1(n) + D_2(n) + \cdots \quad (2)$$

Our first theorem sets up a more refined correspondence between the terms in the right and left members of (2).

THEOREM 1. For all $n \geq 1$, $r \geq 0$, we have

$$U_{2r+1}(n) = D_{2r+1}(n) + D_{2r}(n). \quad (3)$$

Our second theorem is a paraphrase of the double identity

$$\begin{aligned} 1 - \frac{x}{1+x} + \frac{x^3}{(1+x)(1+x^3)} - \frac{x^6}{(1+x)(1+x^3)(1+x^6)} + \dots \\ = 1 - x + (1+x)x^2 - (1+x)(1+x^3)x^5 + (1+x)(1+x^3)(1+x^6)x^8 - \dots \quad (4) \\ = 1 - x + x^2 - x^5 + x^7 - x^{12} + x^{15} - x^{22} + \dots, \end{aligned}$$

the exponents in the last series being the pentagonal numbers $\frac{1}{2}(3k^2 \pm k)$.

Definition 4: $Q_a(n)$ = the number of partitions of n into distinct parts, the maximum part being $\equiv a \pmod{2}$, $a = 0, 1$.

Definition 5: $Q_b^*(n)$ = the number of partitions of n into odd parts, the maximum part being $\equiv b \pmod{4}$, $b = 1, 3$.

THEOREM 2.

- (i) $Q_1^*(2n) = Q_0(2n)$; $Q_3^*(2n) = Q_1(2n)$.
- (ii) $Q_1^*(2n+1) = Q_1(2n+1)$; $Q_3^*(2n+1) = Q_0(2n+1)$.
- (iii) $Q_0(n) - Q_1(n) = +1$ if $n = \frac{1}{2}(3k^2 + k)$, $k \geq 0$,
 $= -1$ if $n = \frac{1}{2}(3k^2 - k)$, $k > 0$,
 $= 0$ otherwise.

Part (iii) of Theorem (2) bears some resemblance to the famous pentagonal number theorem of Euler, but we have not been able to establish any real connection between the two theorems.

Definition 6: $p(n)$ = the number of unrestricted partitions of n ; $p(0) = 1$; $p(n) = 0$ for $n < 0$.

Definition 7: $P_r(n)$ = the number of partitions of n with rank r ; $P_0(0) = 1$; $P_r(n) = 0$ for $r \neq 0$, $n \leq 0$.

THEOREM 3. For $k > 1$, $r > \max(0, k-5)$,

$$P_r(r+k) = p(k-1) - p(k-2). \quad (5)$$

THEOREM 4.

- (i) $P_0(n+1) + P_0(n) + 2P_3(n-1) = p(n+1) - p(n)$ ($n > 0$).
- (ii) $P_0(n-1) - P_1(n) + P_3(n-2) - P_4(n-3) = 0$ ($n > 1$).
- (iii) $P_0(n) - P_1(n-1) - P_2(n-1) + P_3(n-2) = 0$ ($n > 1$).
- (iv) $P_{r+1}(n) - P_r(n-1) - P_{r+3}(n-r-2) + P_{r+4}(n-r-3) = 0$
 $(r > 0, n > 0)$.

We observe that (i) enables us to determine the unrestricted partition

function from the two functions $P_0(n)$ and $P_3(n)$. In fact, if we sum (i) for $1 \leq n \leq N$, we obtain

$$p(N+1) = P_0(N+1) + 2P_0(N) + 2 \sum_{n=1}^{N-1} (P_0(n) + P_3(n)). \quad (6)$$

Equations (ii), (iii), (iv), together with the obvious remark that $P_r(n) = P_{-r}(n)$, yield the result that the functions $P_r(n)$ can all be determined from the functions $P_0(n)$, $P_1(n)$, $P_2(n)$.

Definition 8: $L(n)$ = the number of partitions of n into distinct parts, the minimum part being odd.

THEOREM 5. *For $n \geq 1$, $L(n)$ is odd if and only if n is a square.* It is possible to obtain more precise information about the arithmetic properties of $L(n)$ for special forms of n . We shall restrict ourselves to the remark that

$$L(p^{2m}) \equiv 1 \pmod{4}$$

if p is a prime.

¹ To the best of our knowledge, this concept was first introduced by F. J. Dyson in the *American Mathematical Monthly*, August-September, 1947, p. 418.

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